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Editorial

A change of management

From 1 April 1992 an unannounced change occurred within the organisation of the journal which was doubtless noticed by those of our readers who regularly scan the cover and title pages. Mr Redmond Smith retired as editor and the burden of responsibility is now mine. 'Red', as he is affectionately known to his friends and colleagues, has been associated with the journal for over 30 years, eight of them as editor. During that time many changes and improvements have been introduced to both the format and the content of the journal. A notable feature has been the 'mini review' which many readers have found valuable. As editor, Red was tireless in his efforts in obtaining a fair review of submitted articles and regarded it as his responsibility to ensure that each article was relevant to the aims of the journal and of interest to the readership. Accordingly there was a strong commitment to the judicious editorial. During his period as editor, the publication time for papers has recently been reduced to six months despite an ever increasing number of submitted articles. Redmond conducted the business of the journal with calm and equanimity, and on behalf of the readers, I would like to extend to him our warmest thanks.

Of course with a new editor there are new ways of conducting business. While the aims of the journal will remain broadly the same, namely to foster dialogue in clinical ophthalmology and to reflect current opinion in ophthalmology generally, new ideas will be especially welcome. Plans for the journal are still in the formative stage, but considerable thought is being given to ways of raising the profile of investigative ophthalmology within its pages. In addition, a new format for the case report might be appropriate. These are only a few of the concerns for the editor and his board and feedback from the readership would be welcome.

A change in editorship is an opportune moment to ask what we want from a journal. In the case of the *BJO*, it is time we looked further afield for answers. Ophthalmology is very much an international activity with the flow of ideas rapidly crossing national boundaries. For this reason the editorial board has been expanded to include members from Europe, Australia, and North America, and they, with the other new UK members of the board, are welcomed. Hopefully, this will ensure that the international flow of ideas through the pages of the journal is always in multiple directions.

There are many current journals relating to ophthalmology and new ones appear every year, often with a highly specialised field of interest. This may reflect increased levels of research and clinical activity, but it may also indicate that highly specialised articles have difficulty in finding a place in the more general ophthalmic journals. The main criterion of acceptance for journal articles, however, should rest on the quality of the work reported, and journals of general ophthalmology should provide appropriate space for highly specialised material, as indeed many already do. This encourages high quality research to be undertaken especially by our younger ophthalmic colleagues and those in training and, in the end, reaps benefits for the specialty.

The *BJO* has a long and illustrious past and has been served well by many, particularly its most recent editor. Its future is dependent on new blood and new ideas which will carry it forward into the next century. A sign of health is the ability to respond to and to initiate change and hopefully these changes can be made by building on the achievements of the past.

J V FORRESTER

A reminder that papers intended for publication should now be sent to Professor Forrester at the Department of Ophthalmology, University of Aberdeen Medical School, Foresterhill, Aberdeen AB9 2ZD, Scotland.

British Journal of Ophthalmology, 1992, 76, 386

Editorial

Lacrimal lesions and orbital surgeons

Perhaps one of the factors that influences the decision to pursue a career in ophthalmology is the rarity with which one has to make decisions that could influence life expectancy. That is not the lot of the orbital surgeon, particularly when managing lacrimal fossa lesions. There are few other instances in ophthalmic practice where a wrong decision can be so costly. In this issue the results of the Moorfields orbital clinic's experience in the management of lacrimal gland tumours are reported in two papers by Wright *et al*. It is an extraordinary piece of work: extraordinary because of the size of the series, the length of the follow-up, and, in particular, because the cases have all been managed by one surgeon. It is this type of work that advances knowledge. Yet, as the papers acknowledge, much work remains to be done and many questions remain unanswered.

Make no mistake, the management of lacrimal gland lesions is not easy. There are problems associated with clinical decision-making, the surgery is demanding, and the pathology is difficult. The lacrimal gland has a limited range of pathological responses. However these lesions are rare and indeed the average ophthalmologist could expect to see a new case every 10 years or so. There are many diagnostic pitfalls. Imaging techniques contribute to the management but are not diagnostic. Most of the lesions be they developmental, infective, inflammatory, or neoplastic will shrink with systemic steroids. There is no place for a trial of steroids. For persisting lesions the only decision to be made is whether to carry out an incisional or an excisional biopsy. The main concern is with the mis-diagnosis of a benign mixed cell tumour, carrying out an incisional biopsy, and breaching the false capsule with the associated significant risk of malignant recurrence. Even in the best hands mistakes can occur. The art is in retrieving that situation. Malignant tumours of the gland are aggressive lesions requiring aggressive management. An incisional biopsy is a relatively straightforward procedure while an excisional biopsy, with or without retention of the palpebral lobe of the gland, is technically difficult requiring intimate knowledge of orbital anatomy. The anaesthetist has an important role to play. Hypotensive anaesthesia contributes to a blood free operative field and enhancement of tissue planes. Ultimately the diagnosis is in the hands of the pathologist. On the face of it the reclassification in Wright's paper of two of the malignant tumours as benign seems at best careless. This simply highlights the difficulty the pathologist has to face and this further supports the antagonist's side in the debate over the technique of fine needle aspirational biopsy.

These papers contain clear guidelines on the management of these tumours, based on experience and supported by clearly presented clinical evidence. If ever there was a case for the establishment of recognised referral centres for the management of orbital cases it is contained within these papers. They are to be commended.

P M KYLE

British Journal of Ophthalmology, 1992, 76, 387–394

ORIGINAL ARTICLES

The pattern electroretinogram in glaucoma and ocular hypertension

E O'Donaghue, G B Arden, F O'Sullivan, F Falcão-Reis, B Moriarty, R A Hitchings, W Spilleers, C Hogg, G Weinstein

Abstract

Thirty one eyes with established glaucoma, 61 high risk ocular hypertensive (OHTs) eyes, 66 medium risk OHT eyes, 58 low risk OHT eyes, and 47 control eyes have been followed for up to 2 years by clinical examination and pattern electroretinography (PERG). The study was 'masked' so electrophysiological and clinical data were kept separate. Criteria have been devised which enable PERG measurements to distinguish all established glaucomatous eyes from all normal controls; these criteria demonstrate abnormalities in some OHT eyes, particularly those at high risk. The PERG abnormality is greatest in eyes with established glaucoma in which the intraocular pressure has been lowered by treatment. The PERG becomes smaller as the degree of clinical abnormality increases. Test-retest variability of the PERG is sufficiently low to ensure that most of those first described as abnormal continue to be so.

The Electrodiagnosis and Glaucoma Departments of Moorfields Eye Hospital, City Rd, London EC1
E O'Donoghue†
G B Arden
F O'Sullivan
F Falcão-Reis*
B Moriarty†
R A Hitchings
W Spilleers§
C Hogg
G Weinstein¶

Present addresses:
* Department of Ophthalmology, Oporto Medical School, Av Prof Hernani Monteiro, 4200 Oporto, Portugal;
† Department of Ophthalmology, The Eye Hospital, Manchester, England; § Department of Ophthalmology, KU Leuven, Kapucijnenvor B-3000 Leuven, Belgium;
¶ Department of Ophthalmology, West Virginia University, Morgantown, West Virginia, USA.

Correspondence to:
G B Arden.

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The standard definition of glaucoma (raised intraocular pressure (IOP) changes in the disc and a characteristic field defect) cannot be made until a considerable proportion of the ganglion cells in the retina are dead.[1] However, it is known that by the time the diagnosis is made, the underlying condition of ocular hypertension (OHT) may have been present for up to 10 years[2,3] so the diagnosis records an arbitrary point (determined by the sensitivity of the diagnostic methods) in a continuing decline. There have been two recent reports[4,5] which suggest that treatment of glaucoma suspects, begun earlier, reduces IOP and in follow-up, the rate of development of scotomata decreases, compared with untreated OHT. It is therefore possible that by treating OHT itself, and not waiting for diagnosis by perimetric means, the appearance of scotomata could be largely prevented. The problem with this suggestion is that there are a very large number of patients with OHT, and only a small proportion will develop field defects in the near future.[2,3] Universal treatment is therefore most undesirable. What is required is a means of detection of lesser degrees of functional defect than is possible with perimeters. Some tests proposed to fill this gap (measurement of defects in the nerve fibre layer for example) require considerable skill to record and to interpret.[6,7,8] They record structural retinal or optic nerve damage. It would be desirable to develop functional tests to measure very small changes from normal.

First reports have suggested that this requirement is fulfilled by an objective electrophysiological test of visual function, the pattern electroretinogram (PERG).[9-22] We and others have shown that the PERG is abnormal in patients with established glaucoma. In addition, colour vision tests appear to discriminate between normals and patients with glaucoma.[23,24] What is required is a prolonged and careful investigation into the progress of the disease as monitored by these tests in a larger cohort. The aim to establish whether the tests can be used in the management of patients with various grades of OHT. We report below on results with the PERG (another paper describes results obtained by testing macular colour vision).[25]

Methods

RECRUITMENT

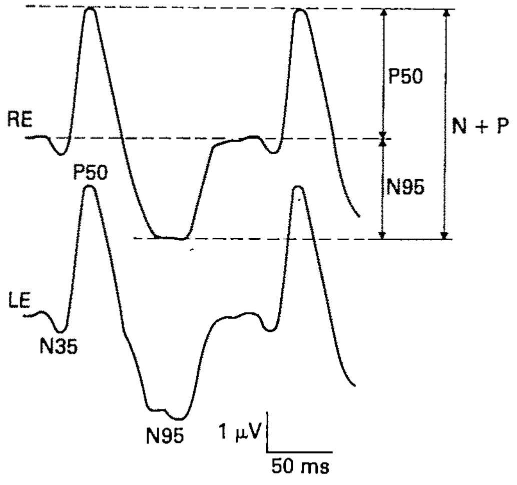
We aimed to recruit approximately equal numbers of patients with established glaucoma, high risk, medium risk, and low risk glaucoma suspects, and normal subjects. The glaucoma patients were recruited from the glaucoma clinic: all eyes had typical reproducible glaucomatous field defects. The glaucoma suspects were classified as high, medium, or low risk according to the level of their IOP and cup:disc ratio (CC:D) (see Table 1 for exact criteria). Yablonski has shown that these groups have different rates of conversion to glaucoma and that the higher the IOP and the C:D ratio the greater is the risk of developing glaucoma.[26] Recruitment of patients with established glaucoma and glaucoma suspects was performed by a clinical fellow who was not concerned with the PERG testing. The normal subjects were recruited from patients'

Table 1 Criteria for classification of glaucoma suspects

IOP (mm Hg)			*C:D ratio*	*Perimetry*
Normals	<21		<0·6	Normal
Low risk	<26		<0·6	Normal
Medium risk	>26	or	>0·6	Normal
High risk	>26	and	>0·6	Normal
Glaucoma	Any		>0·6	Abnormal

Abnormal perimetry was defined as 3 or more adjacent points depressed by 5 db or more and reproduced on 3 occasions.

Figure 1 Diagram of normal transient PERGs illustrating how measurements of negative and positive waves were made.



spouses, hospital staff, and subjects referred to other hospital departments with minor uniocular complaints and essentially normal examination results. Thus, one eye was chosen from each normal subject for testing.

EXCLUSIONS

Patients were excluded if they suffered from other eye disease or severe systemic disease. Patients' eyes with visual acuity <6/9 were excluded: these failures were due to cataract, amblyopia, or other uniocular conditions. Four individuals who failed to attend the follow-up were also excluded. By December 1990, 31 glaucoma eyes from 21 subjects, 61 high risk eyes from 41 subjects, 66 medium risk eyes from 43 subjects, 58 low risk eyes from 33 subjects, and 47 normal eyes from 47 subjects had been recruited to this study and examined as described below over a minimum period of 1 year. Sixty eyes from 138 patients were excluded on the grounds indicated above.

Table 2A Mean values of four PERG parameters for the right eyes of glaucoma patients, high, medium, and low risk suspects. Also mean values of PERG parameters in 47 normal eyes

Class	*Eyes (n)*	*Subjects (n)*	*Age*		*P50*	*N95*	*N/P*	*N+P*
Glaucoma	15	15	Mean	56	1·53	0·23	0·16	1·77
			SD	15	0·66	0·28	0·21	0·73
High risk	30	30	Mean	55	2·29	0·94	0·39	3·26
			SD	10	0·80	0·74	0·26	1·33
Medium risk	31	31	Mean	54	2·15	1·18	0·57	3·32
			SD	10	0·79	0·77	0·35	1·28
Low risk	30	30	Mean	56	2·54	1·08	0·44	3·66
			SD	11	0·89	0·78	0·34	1·25
Normals	47	47	Mean	41	2·97	1·39	0·48	4·36
			SD	11	0·77	0·62	0·21	1·12

NB The ratios N/P are the means of the individual values; they are not therefore identical to the ratios of the means shown in the columns to the left. Also note that only one eye of each normal subject was tested; therefore the normal values are identical in Tables 2A and 2B.

Table 2B Mean values of PERG parameters for the left eyes of glaucoma patients, high, medium, and low risk suspects. Also mean values of PERG parameters in 47 normal eyes

Class	*Eyes (n)*	*Subjects (n)*	*Age*		*P50*	*N95*	*N/P*	*N+P*
Glaucoma	16	16	Mean	55	1·43	0·13	0·09	1·57
			SD	14	0·47	0·16	0·12	0·53
High risk	31	31	Mean	55	2·09	0·64	0·31	2·77
			SD	10	0·91	0·52	0·27	1·13
Medium risk	35	35	Mean	56	2·21	1·24	0·61	3·45
			SD	12	0·80	0·79	0·52	1·27
Low risk	28	28	Mean	56	2·49	0·98	0·40	3·47
			SD	11	0·76	0·84	0·36	1·17
Normals	47	47	Mean	41	2·97	1·39	0·48	4·36
			SD	11	0·77	0·62	0·21	1·12

NB The ratios N/P are the means of the individual values; they are not therefore identical to the ratios of the means shown in the columns to the left.

CLINICAL TESTS

All patients were examined every 6 months. At each visit visual acuity, applanation tonometry, and full clinical examination of fundus and disc were carried out. Perimetry was carried out with the Humphrey perimeter, program 24/II, and analysed statistically. In all cases disc photographs were taken annually both in full colour and in red-free light. A fasting blood glucose test was performed on the first visit to identify any subject with diabetes mellitus. If this diagnosis was subsequently confirmed, the patient was excluded from the special tests.

SPECIAL TESTS

Patients were referred to the electrodiagnostic department for PERG testing. No clinical information was sent with the patients. The technique for recording PERGs has been described previously.[9 17 22] The PERG was recorded bilaterally, using gold foil corneal electrodes and a reference electrode on the skin near the lateral canthus. The stimulus subtended 16×22° and the pattern reversing checks were of 30′ subtense and near 100% contrast. There were six reversals per second. About 250 sweeps were averaged for a response; four or more responses were recorded. The initial corneal positive response (P50) and the later cornea negative response (N95) were measured separately (see Fig 1). In addition, in many of the patients' PERGs were recorded with reversals every 60 ms to obtain a 'steady state' response, which approximates to a sine wave.[21 27] The time base was lengthened to 180 ms, so three consecutive cycles of response could be visualised during the averaging. Records were obtained with the whole screen exposed (16×22°) and also with an 11° opaque disc placed at the screen centre to remove the stimulus from the central 27% of the retina previously stimulated. Colour contrast sensitivity tests were carried out as described in the accompanying paper.[25]

Tests were carried out by technicians who measured the results. This was cross checked by one of us, who also made the (rare) decision that some results had to be discarded. This occurred if there was inadequate documentation, or internal evidence of inconsistency and poor recording: the most common causes of the latter are mains artefacts and epiphora. Electrodiagnostic reports were entered into the patients' files but were not used in management. Results were entered into a database by the technicians, and again these were rechecked, and a number of anomalous results found to be due to entry errors. At a different time, clinical results were entered, under the supervision of the clinical fellow and entry errors removed by rechecking. Although the clinical fellow was aware of the relationship between all results, apart from this the study was masked and decisions about treatment of patients' eyes were made by persons other than those involved in the tests.

In an attempt to quantify the reproducibility

Table 3 Means and significance of differences between the results from the right eyes of all the patients and normals in the cohort, separated by risk class

Category		*Low risk*				*Medium risk*				*High risk*				*Glaucoma*			
Component		*P50*	*N95*	*(N+P)*	*N/P*	*P50*	*N95*	*(N+P)*	*N/P*	*P50*	*N95*	*(N+P)*	*N/P*	*P50*	*N95*	*(N+P)*	*N/P*
Normal	*t*	1·80	1·57	2·04	0·38	3·77*	1·04	3·07*	0·88	3·10*	2·32*	3·15*	3·79*	6·05*	7·02*	8·18*	4·63*
	df	52	51	51	51	55	55	55	55	54	54	54	54	40	40	40	40
Low risk	*t*					1·78	0·49	1·02	1·43	1·14	0·69	1·17	0·63	3·99*	4·24*	5·56*	3·00*
	df					61	60	60	60	61	59	59	59	46	45	45	45
Medium risk	*t*									0·69	1·24	0·18	2·27	2·71*	4·83*	4·53*	4·34*
	df									64	63	63	63	50	49	48	48
High risk	*t*													3·29*	3·74*	4·20*	3·07*
	df													49	48	48	48

*Indicates that a *t* test between the mean value for this group and the one in the left-hand column shows a difference with a probability $p<0·02$.

of the PERG, in 40 eyes additional PERG recordings were made within 4 weeks of the first. The eyes thus tested were drawn in equal numbers from all the groups described above. The conditions for testing the time of day and the technique were the same at each visit. The technicians conducted the test at random and were unaware of the previous results.

Results

The cohort investigated and the results of the first test are shown in Tables 2A and 2B. The mean age of the established glaucoma patients and glaucoma suspects was 55 years: the mean age of the normals was 41. The small difference in the ages will make no significant change to the results. It has been shown that below the age of 70, the PERG amplitude changes little with age.[27, 28] The PERG is a biphasic response with a cornea positive wave peaking at around 50 ms after the stimulus and a cornea negative wave at about 95 ms. We have derived four parameters from the recording, – that is, P50 (µV), N95, (µV), the total PERG amplitude (N+P), and the ratio N/P. For eyes with established glaucoma the mean values of all these parameters are smaller than for normals. In each of the glaucoma suspect groups, the mean P50, N95 and (N+P) are smaller than for normal subjects. The difference between the normal eye values and the values of the eyes in the suspect groups is greatest for the high risk and is least for the low risk group. The mean results and standard deviations for cohort using all the parameters of the PERG are shown in Tables 2A and 2B. Table 2A shows the results for right eyes only and Table 2B shows those for left eyes only. A statistical analysis was carried out on the results obtained from the right eyes of all patients in the cohort (Table 3). Table 3 shows the results for each parameter of the PERG tested. In this table *t* tests have been done between each group and the starred values are highly significant. Eyes with established glaucoma have PERG parameters which are significantly different from all the other groups of eyes tested. The '*t*' values are very high. For the high risk suspect eyes, all parameters of the measured PERG differ highly significantly from normals and the highest *t* value corresponding to the most significant difference is for N/P. For medium risk eyes, both P50 and (N+P) are significantly different from normal values. The *t* value for (N+P) in low risk eyes is different from normal eyes at the 5% level of significance.[29] An analysis corresponding to that shown in Table 3 was also performed for the data on left eyes, with similar results. Left eye results are not given in Table 3 because of this. The differences between the grouped data for right and left eyes were small, providing the data related to the risk class. The largest differences between the mean values for right and left eyes were seen in the high risk suspect groups, but they were not significant using *t* tests. (For P50 $t=0·88$ and for (N+P) $t=1·55$, $df=62$).

Thus, in grouped data, the amplitude of the PERG reflects the progress of the condition. It should be noted that for the established glaucoma eyes the mean IOP is 17·4 (SD 3·76) mm Hg, lower than in the low risk OHT group (18·7 (SD 5·7)). This would be expected since all glaucoma patients were treated. In the high and medium risk OHT patients, some are treated, but in most cases pressure is considerably raised. The mean level is 24·0 (SD 4·8) mm Hg. The results therefore indicate a correlation between glaucomatous damage and the PERG amplitude, rather than a correlation between increase in pressure and the PERG, or some other interfering variable such as poor vision hindering cooperation in the glaucoma patients.

In persons with asymmetrical glaucoma, the PERG parameters differ in amplitude but within a risk class the results from the right and left eyes of this cohort were very similar, which suggests that the results from the two eyes are largely independent and related to the severity of the disease. The analysis shows that the reduction in PERG parameters progresses with risk factors.

In order to assess which of the PERG parameters are most effective at detecting glaucomatous damage, scatter diagrams plotting N95 versus P50 (N+P) versus N/P and (N+P) versus N/N+P for 47 normal eyes and 31 glaucoma eyes were constructed (Figs 2, 3, and 4 respectively). Figure 2 shows that P50 alone is quite a good discriminator of glaucoma although there is some overlap between normal and glaucomatous eyes. Better discrimination between normal eyes and glaucomatous eyes is seen in Figure 3. The parameter (N+P) allows better separation of the two populations than P50 alone. If a value of (N+P) of 2·7 µV is chosen 29 of 31 glaucoma eyes have (N+P) values below this and all the normal eyes have (N+P) values above. The ratio of N/P is not such a good discriminator, although there is a definite trend for glaucomatous eyes to have smaller N/P values than normal eyes. A more mathematically correct ratio (N/N+P) was chosen and plotted against (N+P) in Figure 4. However this ratio was no better a discriminator than N/P. In summary, in this cohort, the parameter (N+P) allows the best

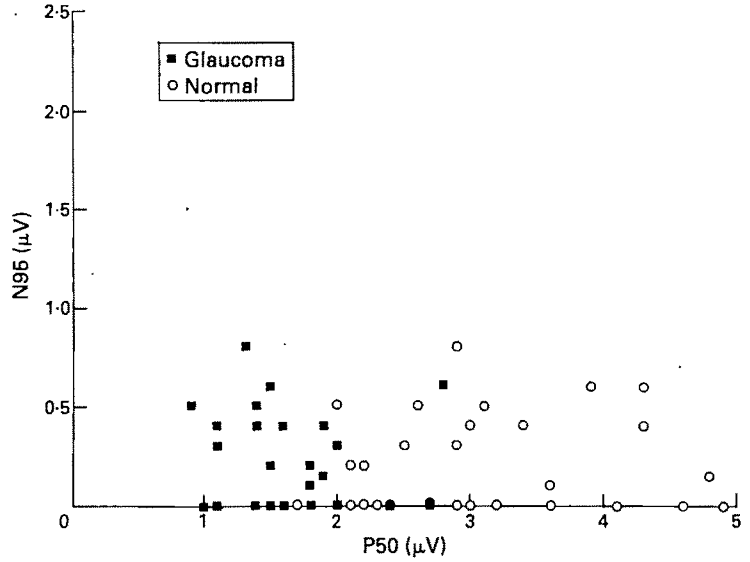


Figure 2 Discrimination of normal eyes from glaucomatous eyes. Scatter diagram of P50 values versus N95.

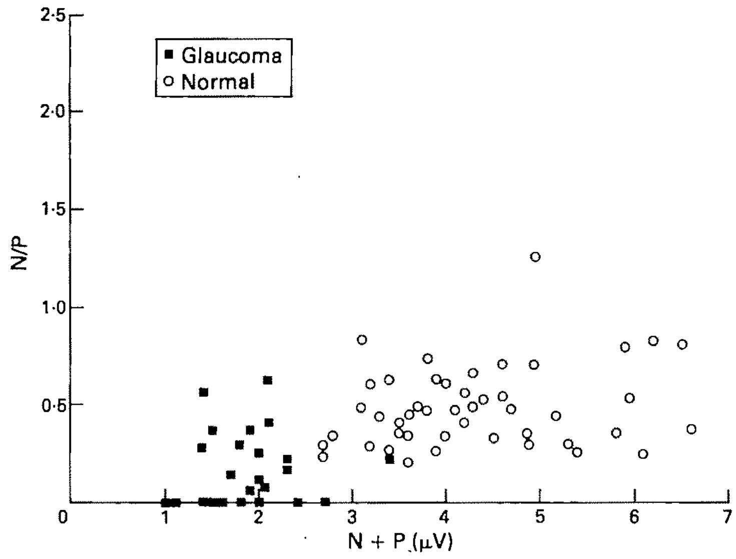


Figure 3 Discrimination of normal eyes from glaucomatous eyes using N+P versus N/P as parameters.

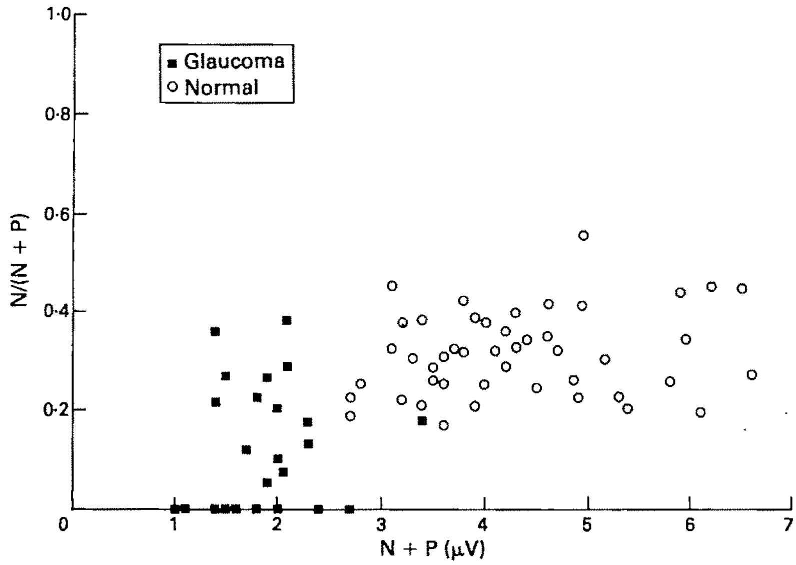


Figure 4 Discrimination of normal eyes from glaucomatous eyes using N+P and N/(N+P) as parameters.

discrimination between glaucomatous and normal eyes.

Table 4 shows the mean PERG parameters for each group of eyes for the first and second visits. The second test occurred 6 months after the first and these second test results might be expected to give an indication of progression over time. It can be seen that the differences between the groups of eyes which were apparent on the first test are reproduced on the second test. In the high risk suspect eyes and established glaucomatous eyes, there is an overall decrease in the mean amplitude of the PERG. It can be seen that there are very small differences between the successive visits. For the medium and low risk groups the largest proportional decline is in the N95 measurement, indicating this may be preferentially affected in the early stages of OHT and glaucoma.

RETINAL AREA TESTED

Following Watanabe *et al*,[30] we recently investigated the central and paracentral components of the PERG in our cohort, using 'steady state' PERGs. Sixty six persons gave useful results on 130 eyes. These results were obtained between August and December 1990. Table 5 shows that the differences described between risk classes for the transient PERG are reproduced for the steady state ERG. The ratios between steady state and transient voltages are not significantly different for any class. The contribution of the central 11° of the retina is about equal to that of the remaining 75% of the screen, for each risk class. The apparent relative increase of the surround response in the glaucoma patients is not significant. Watanabe *et al*[30] who dealt with a smaller sample, and compared controls to eyes with established glaucoma, obtained a different result: the response from the peripheral retina decreases more with disease. Our own work, with luminance and colour contrast sensitivity,[23 31] suggested to us that the peripheral retinal PERG would be more affected in the glaucoma patients than others: however, this expectation has not been fulfilled. A possible explanation is that the amplitude of the PERG is so small in glaucoma, especially when the macula and perimacular regions are not stimulated, that the contamination of the responses by noise becomes more important.

INDIVIDUAL RESULTS

It can be seen in Tables 2 and 5 that the standard deviations of the PERGs are about 25% of the mean values. It was decided to assess carefully the reproducibility of the PERG. Test-retest investigation of the PERG was carried out by repeating tests on 40 patients' eyes within 4 weeks of the first test. The eyes thus tested consisted of equal numbers from each of the three groups and from normal and glaucoma groups. On the first occasion, 16 eyes had abnormal PERGs, and 24 normal responses. The definition of abnormality was (N+P)<2·7, a criterion derived from analysis of all the PERGs recorded, which is further explained in the next section. The mean (N+P) for the first test was

Table 4 A comparison of the results obtained for various PERG parameters (in µV) in first and second visits. (Note the small decreases that occur on the second visit, showing further departures from normal)

	1st visit		*2nd visit*	
	Mean	*SD*	*Mean*	*SD*
P50				
Glaucoma	1·60	(0·49)	1·61	(0·45)
High risk	2.30	(0·69	2·10	(0·60)
Medium risk	2·24	(0·71)	2·05	(0·57)
Low risk	2·56	(0·71)	2·41	(0·65)
N95				
Glaucoma	0·24	(0·28)	0·20	(0·19)
High risk	0·75	(0·58)	0·66	(0·60)
Medium risk	1·19	(0·78)	0·83	(0·60)
Low risk	1·04	(0·78)	0·91	(0·70)
N/P				
Glaucoma	0·14	(0·17)	0·13	(0·16)
High risk	0·35	(0·27)	0·35	(0·37)
Medium risk	0·57	(0·37)	0·45	(0·34)
Low risk	0·44	(0·34)	0·41	(0·34)
(N+P)				
Glaucoma	1·84	(0·65)	1·82	(0·45)
High risk	3·12	(1·08)	2·68	(0·90)
Medium risk	3·44	(0·77)	2·89	(0·88)
Low risk	3·60	(0·88)	3·31	(1·03)

2·98 (SD 1·21) µV and for the second, 3·0 (SD 0·97) µV. The large standard deviations reflect the inter-individual differences in PERG amplitude. The average difference between the two successive readings was 15% of the mean. Eighteen eyes gave smaller responses on the second visit, and 19 larger: in the remaining three, the recordings were identical within the precision of measurement (0·1 µV). In 37 of 40, the difference between the two readings made no difference between the classification of normal and abormal. In the remainder, in two cases, one of the PERG records was borderline normal/abnormal. In the other case the response amplitude appeared to increase from 1·8 to 3·9 µV. The test-retest results are illustrated on a scatter diagram (Fig 5). This illustrates the good correlation between the two sets of values. The regression coefficient=0·80. The formal calculation of the reliability coefficient (1-[square of SD of differences]/square of SD of first measurement)=0·82. Thus test-retest variability should not cause significant differences in the results.

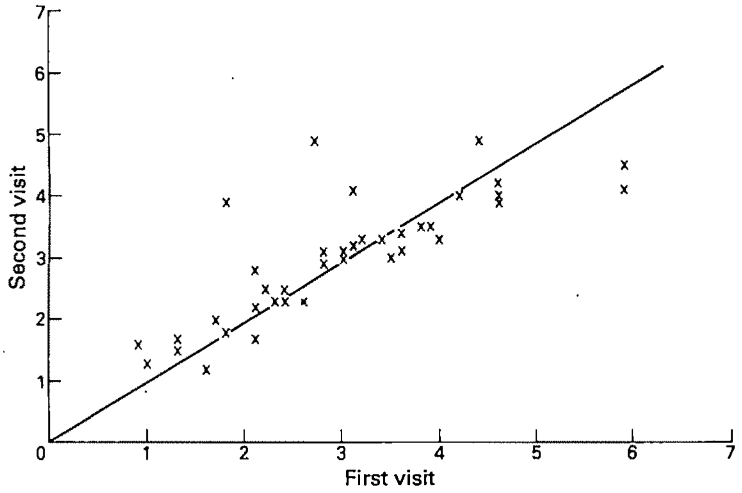


Figure 5 Scattergram illustrating results of N+P values (in µV) for test-retest PERGs on 40 eyes. Regression coefficient of solid line=0·80.

Table 5 A comparison of transient and steady-state PERG amplitudes (in µV) in a selected population

Class	*Number of eyes*	*Number of patients*	*Transient (N+P)*	*Steady state*	*Steady/transient*	*Surrounding/total*
Glaucoma	10	5	1·58	1·22	0·78	0·64
High risk	42	21	3·09	1·97	0·64	0·52
Medium risk	57	29	2·95	1·99	0·69	0·47
Low risk	21	11	3·55	2·32	0·70	0·50

The values for (N+P) can be compared with those in the entire cohort (Tables 2 and 4). The ratio between steady state and transients and the voltage response of the 75% of the peripheral retina compared to the total, are shown in the last two columns

The correlation between clinical estimates of normality and estimates derived from the PERG depends upon the PERG criteria used to divide eyes into normal and abnormal groups.

To attack this problem, we sorted out results, using the facilities of the spreadsheet, using various criterion levels to separate 'normal' from 'abnormal'. Results for the entire cohort are shown in Figures 6, 7, 8 and 9. It can be seen that for eyes of patients with established glaucoma, the total peak-to-peak amplitude of the PERG is nearly always lower than for all the normals; for the high risk suspect eyes, the overlap is considerable, and for low risk it is nearly complete. By adopting various criteria for normality, one can alter the sensitivity and specificity of the test, as shown in Table 6. If a criterion of P50<1·8 µV is chosen, 2% of normal eyes will be considered abnormal, but only 69% of eyes with established glaucoma will be detected. More convincingly, if abnormality is defined as N90 <0·6 µV, then 2% of false positives will be detected, and 84% of all glaucomatous eyes will be abnormal.

Better criteria can be obtained if several measurements are made on the PERG. Our data show that for one determination, abnormality is best defined as (P+N)<2·7 µV, and N/P<0·7. Under such circumstances, no eye from the control group is considered as abnormal, and 94% of eyes of patients with established glaucoma are detected.

The same result can be applied to the data from ocular hypertensives, and there it can be seen that a much lower proportion are considered abnormal, and more than twice as many eyes at severe risk have abnormal PERGs as those in lower risk groups.

SUBSEQUENT VISITS

Glaucoma suspects have repeated field tests, and only when a possible defect is subsequently confirmed is it considered that the patient has changed from OHT to established glaucoma. In just the same way, we can combine the results of two or more PERG tests. If abnormality is defined by the PERG falling below (P+N)<2·7 and N/P<0·7 on one occasion during the entire investigation, the results are as shown in Figure 10: the main difference is that now, 100% of normal eyes can be distinguished from 100% of the eyes of patients with established glaucoma.

Discussion

The present results extend and confirm our (and other) prior reports of PERG abnormalities in glaucoma and ocular hypertension. In particular, we have carried out a masked study in a relatively sizeable cohort for up to 2 years, and have been able to observe and correlate changes in the PERG with the clinical condition. The number of suspects and normals tested is larger than in our previous report and the follow-up time is at least 1 year in all cases. In addition, while in previous work patients' results have been compared with previous established normal values, we have attempted to establish which criteria would be of greatest clinical value in our cohort. We have demonstrated that the PERG is persistently abnormal in eyes with established glaucoma. In our cohort, using as a criterion of abnormality (P+N)<2·7 and N/P<0·7, we were able to separate 94% of such eyes from the normal group with one PERG test. With two tests we were able to differentiate 100% of the glaucomatous eyes from normals. Although this sensitivity and specificity is striking and compares well with automated perimetry, it must be treated with some caution. Our sample size is relatively small and our recruitment criteria removed patients with early cataract or macular degeneration who would normally be included in a glaucoma clinic. Nevertheless the result is objective in a sense that a field test is not. It is as easy for the patient and at least in our cohort seems as reliable as perimetry. Therefore it is likely that serial PERGs can have a place in management of glaucoma patients and suspects.

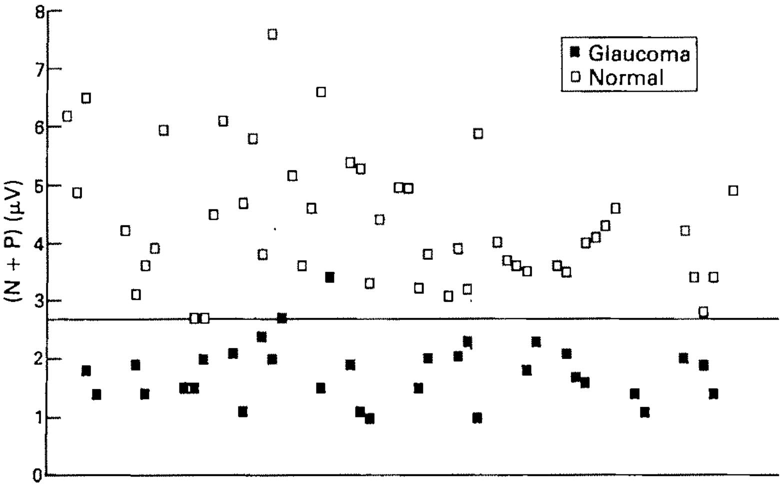


Figure 6 Individual results for normal subjects compared with patients with established glaucoma. The magnitude of (N+P) is plotted on the ordinate. Each interval on the abscissa gives the result from a different eye. Results from both left and right eyes are shown.

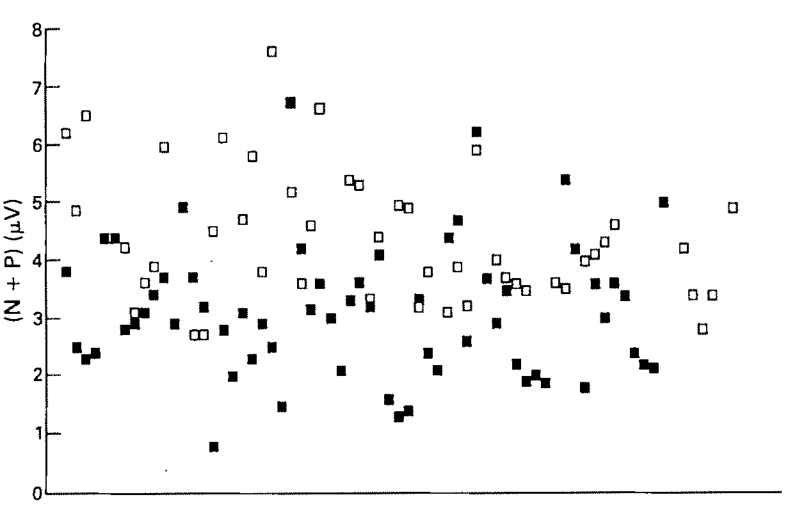


Figure 7 Individual results for normal subjects (open squares) compared with patients in the high risk group (filled squares). Conventions as in Fig 6.

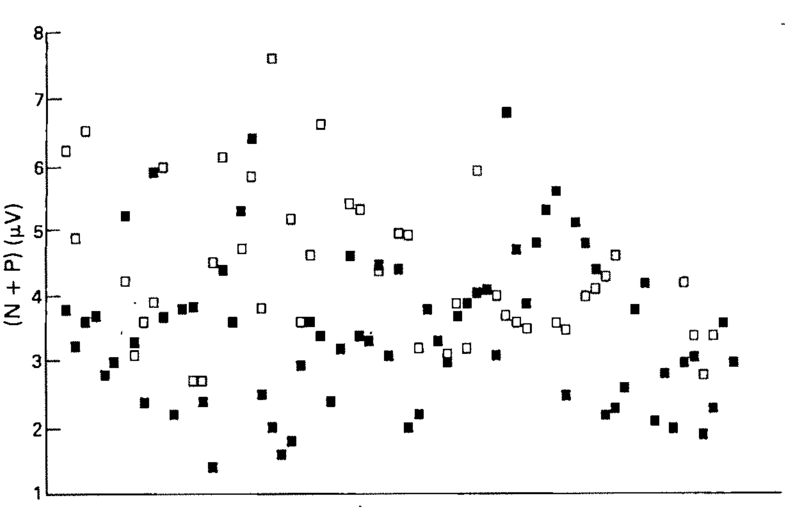


Figure 8 Individual results for normal subjects (open squares) compared with patients in the medium risk group (filled squares). Conventions as in Fig 6.

Our results also demonstrate that glaucoma suspects' eyes show a reduction in their PERG recordings. All our glaucoma suspect groups had smaller mean PERGs than the normals, although this was significant for the high risk group only. The reduction in the PERG of suspects eyes was notable because of the proportionately greater reduction in the N95 component. This had been noted in our previous report and suggests the N95 wave is selectively damaged by the glaucomatous process.

The trial was conducted so that clinical data and the PERG results were collected independently. However the reduction in the PERG mirrored the degree of risk assessed clinically. Thus, high risk suspect eyes had three times as many abnormal PERGs as the low risk eyes; the PERG abnormalities were reproduced on testing at the second visit. This suggests that abnormal PERGs truly reflect abnormal retinal function in glaucoma suspects' eyes. This abnormality was detected while field tests, which are currently used to monitor function, were completely normal. The reasons for the sensitivity of the PERG to retinal or optic nerve damage are not fully understood. Several factors must contribute: the amplitude of the response is due to sampling from a large retinal area, so point-to-point variation in sensitivity is averaged out. Furthermore there is some evidence that the PERG is produced by larger diameter retinal ganglion cells[32] which are known to be affected earlier than small ganglion cells in the evolution of glaucoma.[33] The steady state or peripheral retinal responses in our hands are no more effective criteria, though our experience of them is not so extensive. These results are of interest insofar as they may give some clues about the underlying pathology. For example, the P50 response is affected in glaucoma although it is generated in part by the interneurons or glia of the retina, and only partly by ganglion cells: this suggests that increased pressure may do more than damage optic axons near the cribriform plate.

Although the PERG is an objective test, the voltage is small and the recordings contaminated by noise and artefacts. We have used various methods of stimulation and recording in order to identify which is the most efficient method. However, no one method seems to have any obvious superiority. Our results with test-retest

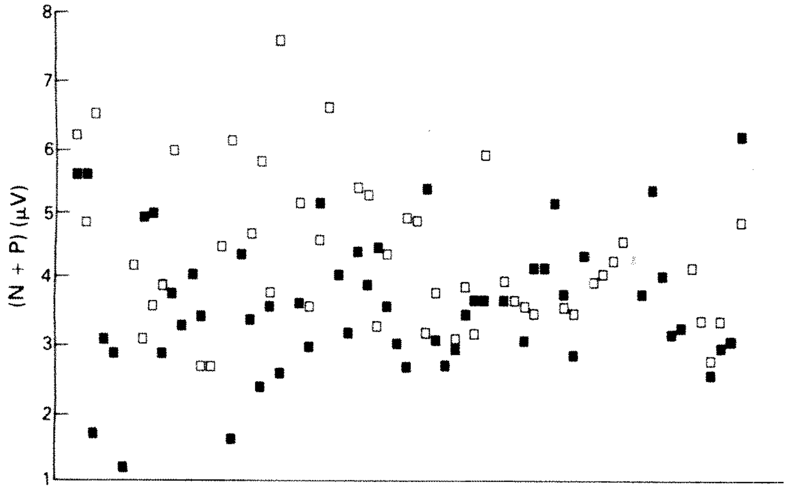


Figure 9 Individual results for normal eyes (open squares) compared to eyes in the low risk group (filled squares). Conventions as in Fig 6.

Table 6 Sensitivity and specificity obtained for various criteria of abnormality of the PERG

	Percentage of eyes considered abnormal, using different criteria				
Criterion	*Controls*	*Glaucoma*	*High risk*	*Medium risk*	*Low risk*
P50					
<2·2	13	90	47	53	20
<2·0	2	88	38	42	15
<1·8	2	69	29	15	17
<1·7	0	69	19	20	11
N95					
<0·8	13	94	50	25	44
<0·7	6	91	43	16	38
<0·6	2	84	34	16	37
<0·5	0	75	33	13	13
(P50+N95)					
<3·0	6	94	55	29	28
<2·7	0	94	43	24	18
<2·5	0	94	38	17	16
<2·3	0	88	30	28	13
P+N<2·7+N<0·9	0	94	34	15	17
P+N<2·7+N<0·7	6	97	65	31	41
P+N<2·7+N/P<0·7	0	94	29	13	13
P+N<2·7+N/P<0·5	0	84	53	29	39

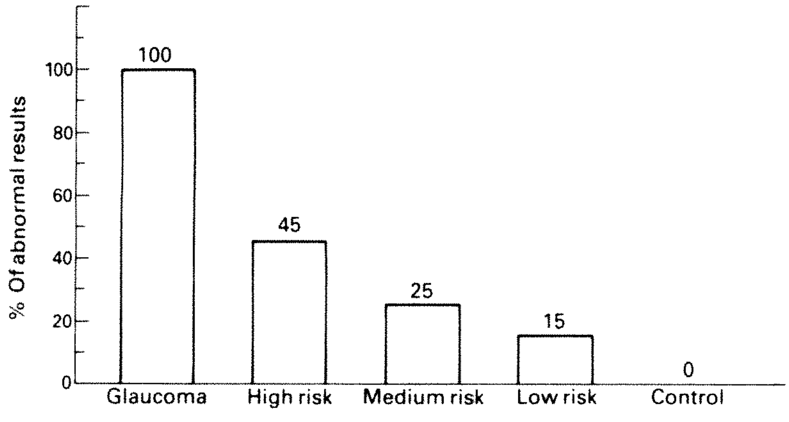


Figure 10 Sensitivity and specificity of the PERG. The criteria for abnormality were that (N+P) should be <2·7 µV, and N/P<0·7 on at least one occasion. The numbers above the bars give the percentage of abnormalities found. Database as for Table 3. The results refer to individual eyes.

show acceptable reproducibility: although there is some variability in each individual's results on repeat testing, this is only very infrequently of a degree which would cause a previously normal result to be subsequently classified as abnormal, or vice versa. We have reported the results of PERG tests on a sizeable cohort followed for 1 year. The follow-up of these patients is continuing; this will provide further information on the natural evolution of glaucomatous disease as monitored by the PERG. It will allow us to correlate the PERG results with the subsequent development of perimetric defects and enable us to determine whether treatment of glaucoma suspects with topical β blockers has an effect on the PERG.

In summary, the PERG is as quick to perform as a test of the visual field with automated perimetry. It is subject to some variability, but this is of an acceptable degree. The PERG convincingly separates all persons with field defects from normals and also demonstrates that a proportion of suspects have reduced responses. The proportion of abnormal PERG mirrors the degree of risk of the suspects. Our experience suggests than an abnormal PERG is an indicator of retinal damage during the phase of 'preglaucoma' before any scotoma can be detected by perimetry. Logic suggests that the PERG could be used for screening in this population, but to demonstrate its value in such a role would require a larger and more prolonged controlled clinical trial.

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Pleomorphic adenoma of the lacrimal gland

Geoffrey E Rose, John E Wright

Orbital Clinic,
Moorfields Eye Hospital,
London EC1V 2PD
G E Rose
J E Wright

Correspondence to:
Mr J E Wright, Moorfields
Eye Hospital, City Road,
London EC1V 2PD.

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Abstract

To reduce the risk of recurrence and malignant transformation, pleomorphic adenomas of the lacrimal gland should be removed intact, without prior biopsy. Seventy one of the 78 patients in this series were referred without previous surgery, and, on clinical or radiological evidence, 63 (89%) tumours were correctly diagnosed and totally excised, with preservation of a margin of the surrounding normal tissue. Sixty three patients had tumour within the body of the gland and 55 (84%) had radiological signs or satisfied clinical criteria for pleomorphic adenoma that we suggested previously; that is, over 1 year of symptoms and absence of pain. Eight (16%) orbital lobe tumours were misdiagnosed preoperatively and biopsied; in all these patients symptoms had been present for less than 1 year, and four patients had pain. Eight patients with tumours arising in the palpebral lobe had a short history of an upper lid mass, and their tumours were excised without biopsy. With the surgical techniques described in this paper, there has been no recurrence of tumour in patients with follow-up as long as 21 years. A modification of surgical technique, with preservation of the palpebral lobe of the gland, has reduced the incidence of postoperative dry eye and distortion of the upper eyelid.

Pleomorphic adenomas of the lacrimal gland have a high rate of recurrence if tumour cells are dispersed into the adjacent tissues during excision or biopsy,[1] and recurrence are often malignant.[1-3] Ideally these tumours should be diagnosed on radiological and clinical evidence and biopsy avoided.

An algorithm for the management of masses arising in the lacrimal gland fossa has been previously proposed by one of us.[4 5] High-resolution computed tomography (CT) can help increase the proportion correctly diagnosed before surgery.[6]

The long-term results for 30 previously reported patients,[5] together with those for a further 48 patients, are described in this paper, and the efficiency of our earlier diagnostic algorithm[4] is examined in relation to increased clinical and radiological experience. The results of a modified surgical technique are also described.

Patients and methods

Between 1968 and 1990, 78 patients (40 male, 38 female) attended Moorfields Eye Hospital with pleomorphic adenoma of the lacrimal gland, representing about 2% of patients attending the Orbital Clinic.

A careful history was taken from all the patients, all underwent ophthalmological examination, and many were reviewed by a neurologist and a physician. Further investigation included orbital B-mode ultrasonography (to 1977), plain radiography or polytomography of the skull or orbits (65 patients), orbital venography (to 1974), or CT (from 1975; available for review for 54 patients).

Most patients have been treated according to our defined clinical algorithm.[4] In practice the patients may be classed into five principal groups:

(I) Patients undergoing resection of palpebral lobe tumours, with intact capsule, generally through a trans-septal approach.

(II) Patients having lateral orbitotomy and resection of tumours arising in the orbital lobe of the lacrimal gland.

(III) Patients who underwent trans-septal biopsy of tumours within the orbital portion of the lacrimal gland.

(IV) Patients undergoing lateral orbitotomy for orbital lobe tumours, together with the removal of the surgical track from previous biopsy (includes group (III) patients).

(V) Patients observed clinically (after excision of tumour at a referring hospital) or treated, after referral, for the recurrence of an incompletely excised tumour.

For tumours in the orbital lobe (groups II and IV) adequate access is possible only through a lateral orbitotomy.

TUMOUR RESECTION FROM THE ORBITAL LOBE OF THE LACRIMAL GLAND

Tumour resection is performed under general anaesthesia, by a modified lateral orbitotomy.[7] The periorbita is elevated from the lateral wall and roof of the orbit; at all times the periorbita is retracted with a malleable brain retractor, the greatest care being taken to avoid a breach of the periorbita. The periosteum is incised inferiorly to the lower pole of the orbital part of the gland and the incision extended anteriorly and posteriorly, to beyond the palpable limits of the neoplasm. The incision is continued superiorly at either end of this incision, and the superior periorbita is incised medially to the upper pole of the gland, thereby mobilising the gland on an intact 'island' of free periorbita. The posterior pole of the gland is mobilised by transection of the neurovascular bundle entering the gland, and the mass is rolled forwards to ease the dissection. At all times the mass is manipulated with malleable retractors, and forceps are never used to handle the lesion. Microsurgical dissection is used to separate the mass from the levator muscle and its lateral horn and to identify the junction between the body of the gland and the palpebral lobe. The gland is transected at this junction, as it curves round the lateral horn of the levator muscle. A margin of normal tissue is always left in contact with the tumour mass, and it is sometimes necessary to sacrifice parts of neighbouring structures.

In the earlier cases of the series dissection was continued anteriorly, inferior to the lateral horn of the levator aponeurosis, and the palpebral lobe and ductules of the lacrimal gland were resected; the resulting conjunctival defect was left open to allow free drainage of fluid from the resection site. In more recent cases, if the mass is located solely within the orbital portion of the gland, the isthmus between the orbital and the palpebral lobes is transected at a site clear of the tumour and the palpebral lobe is preserved.

Where a specimen of tumour has been taken for biopsy, the biopsy track and skin scar are excised, in continuity with a total dacryoadenectomy, the aim being to reduce the risk of recurrent disease at the previous biopsy site.

TUMOUR RESECTION FROM THE PALPEBRAL LOBE OF THE LACRIMAL GLAND

Palpebral lobe tumours are resected through an anterior (trans-septal) orbitotomy, though lateral orbitotomy was used in two cases where the posterior extent of the tumour was unclear. The lacrimal ductules and surrounding conjunctiva are resected, along with a 'cuff' of normal tissues, and the isthmus between the palpebral lobe and the body of the gland is divided. In some cases division of the lateral horn of levator aponeurosis is necessary, being repaired after removal of the tumour.

Table 1 Epidemiology and presenting symptoms of patients with pleomorphic adenoma of the lacrimal gland

Characteristic or symptom	*Group I: palpebral lobe tumours (8 cases)*	*Groups II and IV: orbital lobe tumours (65 cases)*	
		Not biopsied (55)	*Biopsied (10)*
Age at first symptom (years); mean, range	46 (22–79)	47 (17–79)	40 (18–65)
Sex (M:F)	5:3	28:27	4:6
Laterality (R:L)	5:3	35:20	4:6
Symptom duration at 1st ophthalmic consultation (yr); mean, range	0·9 (0·1–1·9)	2·2 (0·1–19)	2·6 (0·1–20) 0·25 (0·1–0·6)*
Proptosis	–	42 (76%)	5 (50%)
Diplopia	–	22 (40%)	4 (40%)
Swelling/lump	8 (100%)	14 (25%)	4 (40%)
Visual change	–	15 (27%)	3 (30%)
Lacrimation	2 (25%)	17 (31%)	1 (10%)
Discomfort	1 (12%)	6 (11%)	4 (40%)
Ptosis	–	8 (15%)	1 (10%)

* Excludes two cases biopsied before referral from other hospitals.

Table 2 Clinical signs and preferred preoperative diagnoses for patients with lacrimal pleomorphic adenoma

Clinical sign	*Group I: palpebral lobe tumours (8 cases)*	*Groups II and IV: orbital lobe tumours (65 cases)*	
		Not biopsied (55)	*Biopsied (10)*
Palpable mass	8 (100%)	53 (96%)	10 (100%)
Relative proptosis (mm); mean, range	0·7 (0–2) (6 cases)	5·7 (0–13) (54 cases)	4·1 (0–9)
Displacement of the globe off axis	1 (12%)	52 (95%)	9 (90%)
Restricted ocular movement	1 (12%)	44 (80%)	8 (80%)
Ptosis	2 (25%)	10 (18%)	2 (20%)
Reduced acuity*	1 (12%)	17 (31%)	3 (30%)
Swollen optic disc or optic atrophy	–	–	2 (20%)
Choroidal folds	–	21/53 (40%)	3 (30%)
Preoperative diagnoses			
Pleomorphic adenoma	7 (88%)	51 (93%)	–
Carcinoma/lymphoma	–	–	8 (100%)
Dacryoadenitis	–	–	4 (50%)
Dermoid or other	3 (37%)	8 (15%)	–

* Reduction of two or more Snellen grades below fellow eye.

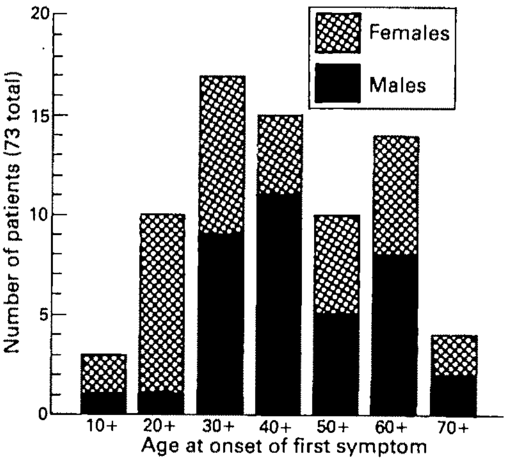

Figure 1 Age (at onset of first symptom) and sex distribution of patients with pleomorphic adenoma of the lacrimal gland.

Results

The age at presentation conforms to a Gaussian distribution (Fig 1) and the incidence is similar in the two sexes (Table 1, Fig 1). However, the duration of the first symptom (Fig 2) and the nature of the presenting symptoms (Table 1) or signs (Table 2) vary for lesions in the different parts of the gland.

CLINICAL PRESENTATION AND MANAGEMENT

Seventy one of the 78 patients (91%) had newly presenting disease, of whom eight (11%) had masses located within the palpebral lobe (Group I). Excision of these tumours, with a clear margin of tissue, was without surgical complication.

Sixty three (89%) of the new patients had tumours in the body of the gland, of which 55 (87%) were considered preoperatively to be pleomorphic adenomas or dermoids (Table 2), having had symptoms longer than a year (69%), an absence of pain (89%), or radiographic changes compatible with a long-standing mass lesion. These 55 tumours were resected intact through a lateral orbitotomy (group II), though in five cases the 'pseudocapsule' of the tumour was thin or adherent to neighbouring bone. Resection of bone was required in five cases and intraoperative spillage of tumour cells possibly occurred in four

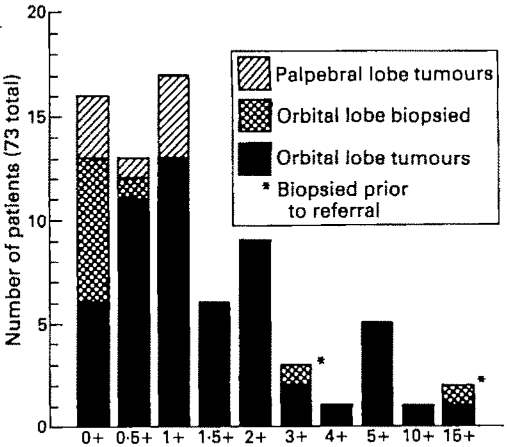

Figure 2 Duration of initial symptom (at first ophthalmic consultation) for patients with pleomorphic adenoma of the lacrimal gland.

Table 3 Surgical resection of pleomorphic adenoma: intraoperative findings and postoperative follow-up interval, complications, and therapy

		Orbital lobe tumours (65)		
		Not biopsied (group II)		
Surgical detail and follow-up	*Palpebral lobe (Group I) tumours (8)*	*Partial adenectomy (26)*	*Total adenectomy (29)*	*Biopsied (group IV) tumours (10)*
Pressure erosion of orbital bone	–	2 (8%)	1 (3%)	1 (10%)
Periosteal breach or attenuated 'pseudocapsule'	–	3 (12%)	2 (7%)	2 (20%)
Follow-up interval from time of treatment (years)				
Mean	1·9	1·5	7·8	6·0
Number ≥5 yr	–	2 (8%)	18 (62%)	4 (40%)
Number ≥10 yr	–	–	12 (40%)	2 (20%)
Number ≥15 yr	–	–	4 (13%)	–
Number ≥20 yr	–	–	2 (7%)	1 (10%)

(Table 3). An intraoperative breach of dura mater occurred in one patient, in whom the orbital roof was eroded by the tumour.

In 8/63 (13%) patients (group III) symptoms were present for less than 1 year and the preoperative diagnosis was malignancy or chronic dacryoadenitis (Table 2). Symptoms had been present for 2 weeks to 7 months at first referral to an ophthalmologist, and half of these patients had pain (in contrast to only six (11%) of group II patients). All patients in group III had tumour biopsy (between 6 weeks and 11 months after their first symptom) and, when histological diagnosis was available, underwent lateral orbitotomy, total dacryoadenectomy, and removal of the biopsy track (Group IV); in two patients the tumour was adherent to the bone of the lateral orbital wall.

Seven patients (9%) had biopsy proved disease prior to referral: two, with symptoms for 4 and 20 years prior to biopsy, underwent lateral orbitotomy with total dacryoadenectomy and removal of the biopsy track (group IV). Tumour recurrence in two patients (at 2 and 4 years after incomplete excision was treated by radical excision and exenteration, respectively. Another patient had a tumour recurrence at 44 years after initial surgery, the resected specimen proving to be malignant. Two patients, referred after incomplete excision of tumour, are being reviewed regularly without further treatment (group V).

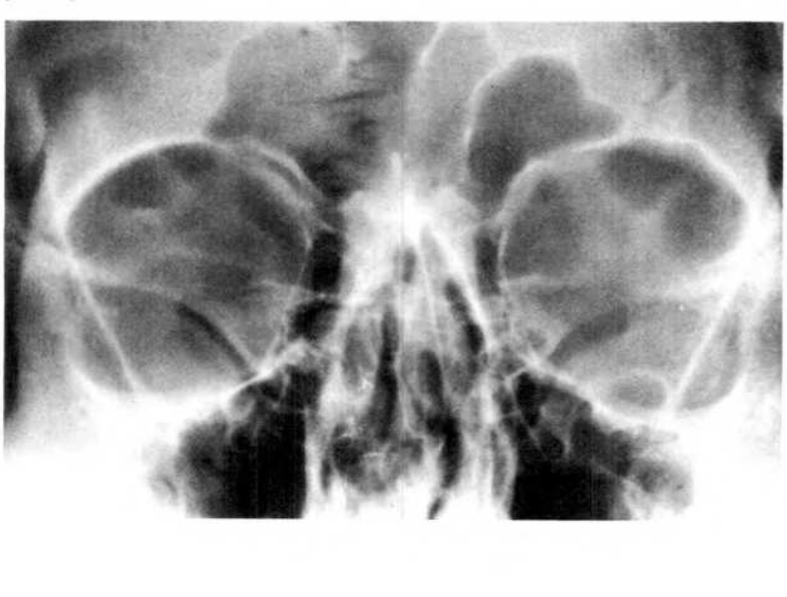

Figure 3 Radiograph of the skull showing expansion of the left lacrimal gland fossa by an orbital lobe pleomorphic adenoma.

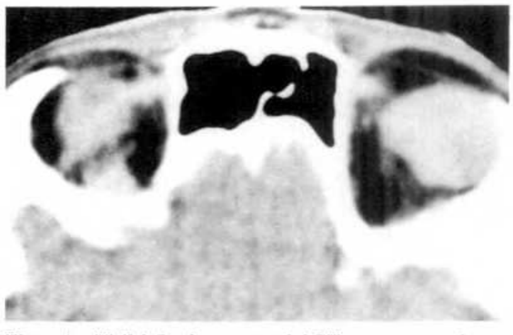

Figure 4 Well defined mass on axial CT scan, commonly seen with pleomorphic adenoma of the orbital lobe of the lacrimal gland.

RADIOLOGY

Orbital lobe tumours caused visible erosion of the bone of the lacrimal fossa in 52/53 patients (98%) whose studies were available for review; it was detectable on plain films (13 cases; Fig 3) or CT (45 cases). Although eight CT studies showed a breach of the orbital roof, this did not display the irregular destruction seen with some malignant neoplasms.

A well defined soft-tissue mass was shown on CT scan in orbital lobe adenomas (Fig 4), but in only few palpebral lobe tumours. Calcification within the body of the gland was visible on four CT studies at soft-tissue windows and was reported pathologically in three (Fig 5A).

EFFECT OF MODIFIED SURGICAL TECHNIQUE ON CLINICAL OUTCOME

With preservation of the palpebral lobe, there was a significant ($p<0·001$) reduction in the incidence of keratoconjunctivitis sicca and the need for topical ocular lubricants (Table 4).

The frequency of other complications is similar in the various groups (Table 4). In the minority of patients experiencing diplopia after lateral orbitotomy, this tended to occur only on extremes of horizontal gaze. Similarly, the degree of ptosis was generally mild, required surgical correction in only five cases.

LENGTH OF FOLLOW-UP INTERVALS

In 43/73 (59%) of patients who underwent primary resection of a tumour the most recent review was in (or after) 1989. Thirty patients who defaulted to follow-up were all free of disease at last clinical examination; many regard the benign tumour as cured, without complication, and hence the relatively high number of defaulters.

The duration of follow-up varies from 0·2–4·7 years for group I, 0·1–21·5 years for group II, and 0·5–19·7 years for groups III and IV (Table 3).

Discussion

Up to half of intrinsic lacrimal gland masses[4 8 9-12] are neoplasms of epithelial origin, of which many are pleomorphic adenomas (benign mixed tumours) derived from the ducts, stroma, and myoepithelial elements of the lacrimal gland.[11]

Although benign tumours, they show cellular cords extending outwards into a 'pseudocapsule,' formed by compression of the surrounding tissues.[14] Resection must therefore be directed towards their intact excision, with removal of an adequate margin of the surrounding tissues.[1 2 4 5 14 15]

OUTCOME AFTER INTACT EXCISION OF ORBITAL LOBE TUMOURS

With an algorithm proposed previously[4] 55/63 (87%) patients were correctly diagnosed preoperatively as requiring a lateral orbitotomy for excision of a benign mass in the body of the lacrimal gland; 69% had symptoms for a year or more prior to ophthalmic consultation, 89% were without pain, and almost all (98%) had radiographic evidence of a well defined mass eroding (rather than invading) the bone of the lacrimal fossa.

There have been no tumour recurrences in this group (Table 3: group II; 55 patients), though the tendency to late recurrence of such tumours precludes a true assessment of 'cure rate' until several decades after treatment.

OUTCOME AFTER BIOPSY AND LATER EXCISION OF ORBITAL LOBE TUMOURS

Orbital lobe tumours were biopsied in eight patients (13%) because of a short history (2 weeks to 7 months) and the presence of pain in four (group III). A short history and pain were considered indicative of malignant change, though, in some, CT displayed features of a benign tumour.[6] Two other patients, both with long histories, were biopsied at the referring hospitals.

All 10 patients underwent lateral orbitotomy, total dacryoadenectomy, and removal of the biopsy track. A limited follow-up for group IV makes assessment of a 'cure rate' impossible, but it is encouraging that there have been no tumour recurrences after as long as 20 years (Table 3).

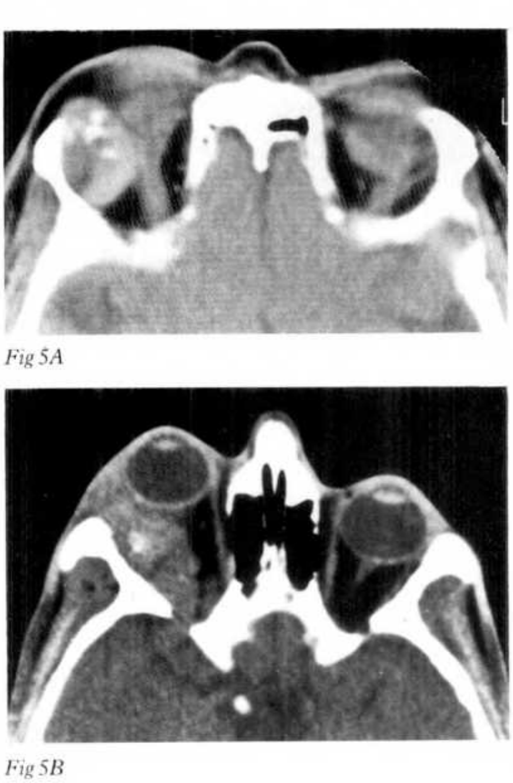

Figure 5 Calcification in pleomorphic adenomas (A) is rare, in comparison with that within lacrimal gland carcinomas (B).

Fig 5A

Fig 5B

The potentially disastrous consequences of the incomplete removal of a pleomorphic adenoma have been emphasised by Font and Gamel,[1] who reported an overall 15-year recurrence rate of 30%. Initial management was critical for prognosis, with a 5-year recurrence rate of 3% if the tumour was totally removed, in contrast to 32% if the tumour was biopsied prior to removal. Recurrent pleomorphic adenoma can undergo malignant change, as in a third of patients first presenting to us with recurrent tumour (group V): Font and Gamel[1] estimate, actuarially, that about 10% of adenomas undergo malignant change by 20 years after first treatment and 20% by 30 years. Recurrence and malignant change of adenomas after incomplete excision are also reported in other series.[3]

Because biopsy of pleomorphic adenomas has such an adverse influence upon the long term outcome, it clearly is preferable that these tumours are diagnosed before surgery and that biopsy is avoided.

CLINICAL AND RADIOLOGICAL DIAGNOSIS OF PLEOMORPHIC ADENOMAS

Diagnosis of palpebral lobe tumours is usually straightforward.[16 17] Patients give a short history (often less than a year: Table 1, Fig 2) of a painless, mobile, hard mass in the lateral aspect of the upper eyelid. High-resolution CT may demonstrate a mass at, or anterior to, the orbital rim and no radiological changes within the orbital lobe of the gland. Excisional biopsy of palpebral lobe tumours, with an adequate margin of normal tissue, is both curative and diagnostic.

Diagnosis of tumours within the body of the lacrimal gland is more difficult. They often present as a slowly progressive mass (Tables 1 and 2) with symptoms such as facial asymmetry (72%) diplopia (40%), or swelling (28%), commonly noted for many years before referral. Lacrimation, often episodic, was a presenting symptom in some patients (Table 1), this symptom being rare in patients with lacrimal gland carcinoma.[18]

The chronic nature of orbital lobe tumours is reflected in often extreme degrees of slowly progressive ocular displacement – here varying, at presentation, from 0 mm to 13 mm of relative proptosis and inferomedial displacement (Table 2). It is also evident radiologically, with erosion of the lacrimal fossa by a smoothly contoured mass present in 70% plain films (Fig 3) and 81% CT studies; the orbital roof or lateral wall was breached in eight cases. There was no evidence of irregular bone invasion or destruction.

Radiological evidence of calcification within benign lesions of the lacrimal fossa is rare[19] and is more common with carcinomas[18] (Fig 5B). In this series diffuse calcification was present on high-resolution CT in three patients and a large nodule of dense calcification – possibly an area of osseus differentiation – was seen in a fourth. Calcification was evident histologically in three tumours.

Table 4 Frequency of late complication (more than 6 months after surgery) in patients undergoing total or partial dacryoadenectomy

Complication	*Palpebral lobe preserved (28)*	*Palpebral lobe excised (45)*	*Significance (p)*
Diplopia extremes of gaze	7 (25%)	14 (31%)	>0·05
Ptosis present	7 (25%)	13 (29%)	>0·05
Ptosis surgery required	1 (4%)	4 (9%)	>0·05
Symblepharon	–	5 (11%)	>0·05
Filamentary keratopathy	7 (25%)	35 (78%)	<0·001
Topical lubricants required	6 (21%)	28 (62%)	<0·001
Occlusion of puncta	–	6 (13%)	0·05

Table 5 Number of patients with pleomorphic adenomas (66) or carcinomas (46) arising in the orbital lobe of the lacrimal gland, classed according to the presence or absence of pain and the duration of initial symptom at the time of first ophthalmic consultation; percentages (in brackets) refer to portions across the rows

	Painless (69)		*Painful (43)*	
Duration of symptoms	*Adenomas (56)*	*Carcinomas (13)*	*Adenomas (10)*	*Carcinomas (33)*
<8 months (45)	11 (24%)	11 (24%)	4 (9%)	19 (43%)
8–12 months (19)	9 (46%)	2 (11%)	2 (11%)	6 (32%)
>12 months (48)	36 (75%)	0	4 (8%)	8 (17%)

Table 6 Management plan for masses within the orbital lobe of the lacrimal gland

	Score	
Characteristic	*−1*	*+1*
Clinical:		
Duration of acute symptoms	<10 months	>10 months
Persistent pain	Present	Absent
Sensory loss	Present	Absent
Radiological:*		
Well-defined round or oval mass	Absent	Present
Moulding of mass to globe or along the lateral orbital wall	Present	Absent
Tumour calcification	Present	Absent
Invasion of bone	Present	Absent
Duration of symptoms in relation to the tumour size	Large tumour; short symptoms	Small tumour; long symptoms

* Features on high-resolution CT studies
Total score: −8 to +2, probably carcinoma (for incisional biopsy); −6 to +2, probably malignant mixed tumour (for incisional or excisional biopsy); +3 to +8, probably pleomorphic adenoma (for excision *without* prior biopsy).

MODIFIED ALGORITHM FOR THE MANAGEMENT OF LACRIMAL GLAND MASSES

The clinical algorithm, proposed previously for the preoperative differentiation of benign from malignant lacrimal gland tumours,[4] may now be modified because of our greater clinical experience[18] and improved radiological imaging.

On grouping the patients according to the presence or absence of pain and the duration of symptoms *at first ophthalmic consultation*, it is evident that the original clinical algorithm[4] is still valid (Table 5); namely, that painful, short-duration lesions[18] favour carcinomas and the converse characteristics favour adenomas. However, we now find that greater discrimination (between carcinomas and adenomas) occurs at 10 months, rather than 12 months, as originally suggested.

Although biopsy was recommended[4] for lesions causing symptoms for less than 1 year, especially if painful (as likely to be carcinoma), we now avoid biopsy if CT shows a smoothly contoured mass expanding the orbit, suggesting a benign lesion.[6] The presence of tumour calcification, significantly more common with carcinomas,[18] favours biopsy.

A modified plan for the management of persistent masses in the lacrimal gland, taking into account the changes in clinical and radiological diagnosis, is given in Table 6. Low scores for the clinical and radiological features favour the diagnosis of carcinoma (for incisional biopsy), whereas higher scores favour pleomorphic adenoma (for intact excisional biopsy).

COMPLICATIONS OF SURGERY AND MODIFIED SURGICAL TECHNIQUE

With the techniques used, intraoperative complications were few. Intraoperative breach of dura mater in one patient was treated by local application of Gelfoam strips and the administration of systemic antibiotics, with uneventful postoperative recovery. If it was considered that the 'pseudocapsule' of the tumour might have been breached during surgery, this was treated by strict surgical isolation, lavage of the operative field after removal of the mass and, where possible, wide excision of neighbouring tissues.

A modified surgical approach for tumours of the orbital lobe, within transection of the isthmus between the orbital and palpebral lobes (and preservation of the latter) provides a significant reduction in corneal surface changes and the need for long-term topical therapy (Table 4). The frequency of persistent diplopia on extreme gaze and ptosis (the latter requiring correction in some cases) was similar in patients after partial or total dacryoadenectomy (Tables 3 and 4); these complications generally result from the lateral orbitotomy and the occasional need for resection of structures neighbouring the tumour, rather than being a direct consequence of the extent of dacryoadenectomy.

At present the numbers of patients free of disease after more than 10 years (14 cases), 15 years (five cases), or 20 years (3 cases) is encouraging. However, only another 10 or 20 years of follow-up will determine whether partial dacryoadenectomy, with its lower complication rate, is a safe treatment for pleomorphic adenomas of the orbital lobe.

Our thanks are due to the many consultant ophthalmic surgeons who referred cases to the Orbital Clinic, to Professor A Garner and his colleagues for the histopathology, to Drs G Lloyd and I Moseley for their radiological skills, and to the staff of the Department of Medical Illustration, Moorfields Eye Hospital, for clinical photography. We are particularly grateful to Dr Ivan Moseley for his most helpful review of the manuscript.

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Primary malignant neoplasms of the lacrimal gland

John E Wright, Geoffrey E Rose, Alec Garner

Abstract

The clinical characteristics and outcome of 50 primary malignant neoplasms of the lacrimal gland are reviewed: 38 (76%) adenoid cystic carcinomas, six (12%) carcinomas arising in pleomorphic adenoma, and six (12%) adenocarcinomas or other types of carcinoma. Most patients presented with a short history and pain, though pain tended to occur less often and later with adenocarcinoma than with adenoid cystic carcinoma. Pain was unrelated to the duration of symptoms, invasion of bone, loss of trigeminal nerve function, or the frequency and time of tumour recurrence. The estimated disease-free survival for patients with adenoid cystic carcinoma was significantly ($p<0{\cdot}01$) reduced where half or more of the biopsy specimen showed basaloid differentiation. Eleven patients underwent extended cranio-orbital resection, and the others received a combination of total dacryoadenectomy and/or radiotherapy. Survival after adenoid cystic carcinomas appears to be significantly ($p<0{\cdot}05$) greater when tumour resection is combined with radiotherapy than after radiotherapy alone. At present, however, the rate of disease-free survival after treatment of adenoid cystic carcinoma appears unaltered by cranio-orbital resection, though these latter patients form a relatively greater proportion of those surviving for more than 10 years. Further long-term follow-up is needed to see if this technique does influence survival.

Primary malignant neoplasms of the lacrimal gland form 1–2% of referrals to specialist orbital clinics[1] and have a high morbidity and mortality.[1-15]

Previous authors have reported that the survival of patients with adenoid cystic carcinoma may be related to the histological type.[16-18] However, the effect of local resection, radiotherapy, or cranio-orbital resection[10-15 18-20] on the frequency or speed of tumour recurrence remains uncertain.

The characteristics and treatment of a group of 24 patients with lacrimal gland carcinomas, grouped according to the histological classification of Foote and Frazell,[21] have been previously reported from this clinic.[15] For this paper, the histopathology of these 24 patients, and of a further 28, has been reviewed and the effect of different clinical managements assessed; two of the original 24 cases were reclassified as benign lesions.

Patients and methods

Between 1968 and 1990, 50 patients attended the Orbital Clinic at Moorfields Eye Hospital with primary malignant neoplasms within the lacrimal gland; they represented less than 2% of about 3500 patients attending this specialist clinic during that period. A further patient, with an adenoid cystic carcinoma arising in an accessory lacrimal gland in the upper conjunctival fornix, is not included in this series.

Case numbers were assigned to each patient, though not in chronological order (Table 1); these correspond to those in a previous paper.[15]

Particular attention was given to the history. All patients underwent a complete ophthalmological examination; they were also reviewed by a neurologist, a physician, and after tissue diagnosis by an oncologist. Further investigation included orbital B-mode ultrasonography (to 1977), plain *x*-radiography of the skull or orbits, orbital venography (to 1974) or *x*-ray computed tomography (from 1974; 42 patients). Magnetic resonance imaging was used in a few patients to define the extent or nature of presenting or recurrent lesions.

All patients had biopsy, generally by a trans-septal approach under general or local anaesthesia. In six cases the lesion was considered to be a pleomorphic adenoma and in-toto dacryoadenectomy was performed.

For the present study the histology of all cases was reviewed by one author (AG), and, where the original diagnosis was either changed or further refined, sections were also examined by Dr Ramon L Font. This review was performed without reference to the clinical presentation or outcome of the patients.

Adenocarcinoma was considered to be of primary lacrimal origin if systemic investigation for tumour was negative and if the patient did not develop another solitary clinical tumour during or after treatment of the lacrimal neoplasm.

TREATMENT METHODS FOR PRIMARY NEOPLASMS OF THE LACRIMAL GLAND

Where the symptoms were prolonged and investigations were suggestive of pleomorphic adenoma,[22] the mass was removed, without prior biopsy, through a lateral orbitotomy (cases 19, 37, 43, 45, 46, 50). In all other cases the lesion was biopsied through a trans-septal approach, thereby maintaining intact periorbita and orbital bones.

Patients in whom the tumour was considered wholly intraorbital underwent en-bloc resection of the orbital contents and the superior and lateral orbital walls.[11 23] At the time of surgery, however, the tumour in cases 2 and 24 was found to extend beyond the orbit.

Most patients received radiotherapy (>50 Gy), and in some cases the bulk of tumour was resected before or after radiotherapy.

If radiotherapy had not been used as primary treatment, recurrent disease was generally

Orbital Clinic, Moorfields Eye Hospital
J E Wright
G E Rose

Department of Histopathology, Institute of Ophthalmology
A Garner

Correspondence to:
Mr J E Wright, Moorfields Eye Hospital, City Road, London EC1V 2PD.

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Table 1 Details, pathology, and treatment of patients with primary malignant neoplasms of the lacrimal gland

Case no	Sex, age	Tumour type	Treatment		Recurrence		Follow-up (years) and outcome
			Date	Therapy	Years	Therapy	
1	F, 14	AC	1976	L+X	9	–	13, Dead*
2	M, 32	AC	1976	O+X	2	X	4, Dead
3	F, 17	AC	1974	X	1	X+C	2, Dead
4	F, 51	AC	1974	X	1	L	3, Dead
5	F, 29	AC	1976	O+X	<1	–	2, Dead
6	M, 64	MM	1977	L+X	1	–	2, Dead
7	M, 49	AC	1976	O			13, Dead*
8	M, 47	A	1977	X	2	–	3, Dead
[9	M, 64	…………… NOW RECLASSIFIED AS PLEOMORPHIC ADENOMA]					
10	F, 57	AC	1980	X	1	–	2, Dead
11	M, 20	AC	1980	X	1	–	2, Dead
12	M, 51	AC	1972	X+O			15, Dead*
13	M, 14	S	1975	L+X			15, Alive
14	M, 55	AC	1974	O			16, Alive*
15	M, 41	AC	1979	X	1	C	1, Dead
16	F, 67	AC	1974	X+L			16, Alive*
17	F, 14	AC	1975	X	<1	—	1, Dead
18	M, 72	AC	1979	X	1	–	2, Dead
19	F, 68	AC	1974	L			6, Dead
20	F, 28	AC	1981	L+X			<1, Alive
21	M, 28	AC	1975	O	7 X,	14 C	15, Alive*
22	F, 28	E	1972	L+X			<1, Alive
23	F, 59	AC	1976	L+X	3	–	4, Dead
[24	F, 22	…………… NOW RECLASSIFIED AS PLEOMORPHIC ADENOMA]					
25	M, 59	A	1985	X	2	–	3, Dead
26	M, 38	MM	1986	O+X			1, Alive
27	M, 62	A	1989	X	1	–	1, Alive
28	M, 45	A	1989	X	1	–	1, Alive
29	M, 78	AC	1982	X	<1	–	1, Dead
30	F, 19	AC	1977	X			6, Alive
31	F, 17	AC	1990	X			<1, Alive
32	F, 37	AC	1986	X			<1, Alive
33	M, 8	AC	1981	L+X+O			7, Alive
34	M, 79	AC	1988	X			<1, Alive
35	M, 29	AC	1987	X			3, Alive
36	F, 35	AC	1985	L	3	–	5, Alive
37	M, 34	AC	1989	L+X			1, Alive
38	M, 23	AC	1988	X+L			2, Alive
39	M, 33	AC	1989	L+X			1, Alive
40	F, 78	AC	1989	X+C	<1	–	1, Alive
41	M, 42	AC	1982	X+O	3	X	6, Alive
42	M, 29	AC	1988/ 1989	X+O	<1	–	2, Alive
43	F, 36	AC	1987	L+X			3, Alive
44	M, 54	AC	1984/ 1986	X+O	4	–	4, Alive
45	F, 59	AC	1989	L+X			1, Alive
46	M, 34	AC	1980	L+X			10, Alive
47	M, 50	AC	1986	O			4, Alive
48	M, 25	AC	1990	X			<1, Alive
49	M, 35	MM	1989	X+O			1, Alive
50	M, 60	MM	1985	L+X	3	O	4, Dead
51	M, 71	MM	1985	X	<1	–	1, Dead
52	F, 50	MM	1985	X	3	–	5, Alive

F, female; M, male; AC, adenoid cystic carcinoma; A, adenocarcinoma; MM, malignant mixed tumour; S, squamous carcinoma; E, mucoepidermoid carcinoma; L, localised resection of tumour; O, en-bloc cranio-orbital resection; X, radiotherapy; C, systemic chemotherapy.
* Denotes patients with adenoid cystic carcinomas surviving for more than 10 years.

Table 2 Patient characteristics and presenting symptoms in 48 patients with malignant neoplasms of the lacrimal gland

Characteristic	*Adenoid cystic carcinoma (38)*	*Adenocarcinoma (4)*	*Malignant mixed tumour (6)*
Sex – M:F	21:17	4:0	5:1
Mean age at onset of symptoms	41 (8–79)	54 (45–62)	53 (35–71)
Patients presenting at 30 years or over	60%	100%	100%
Time from symptoms to consultation (months)	7·6 (0–36)	5·8 (1–9)	38 (1–180)
Time from consultation to biopsy (months)	4·4 (0–33)	2·8 (2–5)	4 (1–180)
Symptoms			
Pain	30 (79%)	2 (50%)	2 (33%)
Globe displacement	18 (47%)	3 (75%)	4 (67%)
Mass or swelling	19 (50%)	4 (100%)	5 (83%)
Diplopia	11 (29%)	1 (25%)	2 (33%)
Visual change	9 (24%)	–	2 (33%)
Ptosis	6 (16%)	1 (25%)	–

Table 3 Frequency of radiological changes

Radiological abnormality		*Adenoid cystic carcinoma (38)*	*Adenocarcinoma (4) and other carcinomas (2)*	*Malignant mixed tumours (6)*
Erosion of bone	PF	21/27 (78%)	3/4 (75%)*	4/5 (80%)
	CT	24/32 (75%)	5/5 (100%)*	5/5 (100%)
Invasion of bone	PF	6/27 (22%)	1/4 (25%)	2/5 (40%)
	CT	11/32 (34%)	1/5 (20%)	3/5 (60%)
Calcification of soft tissues	PT	5/27 (19%)	0/4 (0%)	0/5 (0%)
	CT	7/32 (22%)	3/5 (60%)	4/5 (80%)

PF, plain *x*-rays; CT, *x*-ray computed tomography.
* Includes one squamous carcinoma; all other positive findings were in adenocarcinomas.

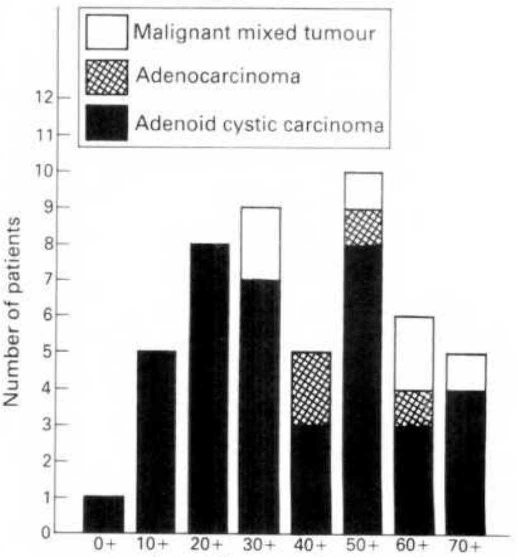

Figure 1 Age of patient at onset of the first symptom of a malignant lacrimal gland tumour.

treated with this therapy. Four patients (cases 3, 15, 21, 40) received chemotherapy for recurrent disease.

Groups were compared by analysis of variance (F), Student's *t* test, the χ^2 statistic, and Fisher's exact (S) test. Kaplan-Meier survival estimates were compared by the Mantel-Haenszel test.

Results

Clinical details were available for all patients, though four patients were lost to follow-up (cases 20, 22, 30, 32).

EPIDEMIOLOGY

Adenoid cystic carcinoma was most frequent (38 cases; 76%), and six patients had carcinoma arising in pleomorphic adenoma ('malignant mixed tumour'), four had adenocarcinoma, one had squamous carcinoma, and one had mucoepidermoid carcinoma (Table 1). In all groups there was a preponderance of males (Table 2).

At onset of symptoms, patients with adenoid cystic carcinoma tended to be younger than those with adenocarcinoma or malignant mixed tumours (Fig 1, Table 2), though this did not reach significance at a 5% type I error (F=1·53, p>0·10). However, all patients presenting under 30 years of age had adenoid cystic carcinoma,

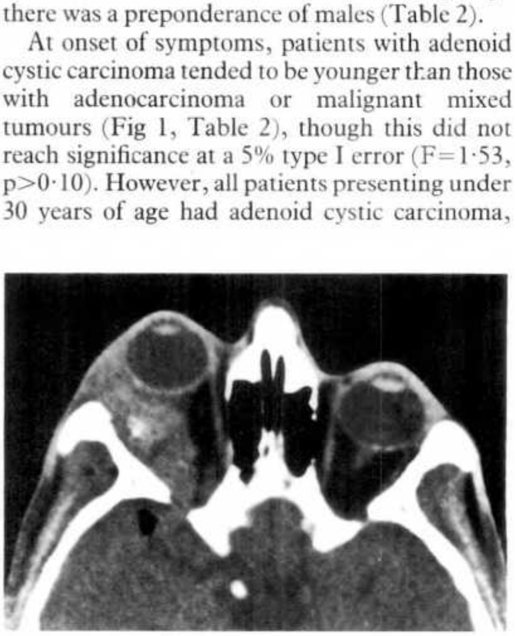

Figure 2 Orbital CT scan showing extraorbital extension of adenoid cystic carcinoma (arrow). The tumour shows a finely-stippled calcification at the soft-tissue settings of this CT scan.

this proportion being significantly ($S=0\cdot04$) higher than that in the older patients (Table 2). Nine (60%) of 15 patients with basaloid adenoid cystic carcinoma presented when over 40 years of age, as compared with 8/23 (35%) of the non-basaloid tumours ($S=0\cdot15$).

SYMPTOMS

The presenting symptoms are given in Table 2. The mean duration of symptoms on first ophthalmic attendance was similar in patients with adenoid cystic carcinomas and adenocarcinomas (Table 2; $t=0\cdot37$, $p>0\cdot70$). However, for both adenoid cystic carcinoma ($t=4\cdot52$, $p<0\cdot001$) and adenocarcinoma ($t=3\cdot93$, $p<0\cdot05$), there was a significant delay between the times of first ophthalmic attendance and biopsy; that is, there was a significant delay in referral for specialist management (Table 2).

The duration of symptoms might influence outcome or treatment. However, for patients with adenoid cystic carcinoma the duration of symptoms *at first ophthalmic attendance* or duration *at the time of biopsy* were *similar* in 18 patients with and 20 without recurrent disease ($p>0\cdot40$ for 'first attendance' and $p>0\cdot50$ for 'time of biopsy') and in 11 patients considered suitable for cranio-orbital resection or 27 cases for whom only radiotherapy and/or local resection was appropriate ($p>0\cdot40$ and $p>0\cdot20$).

CLINICAL SIGNS

All 50 patients had signs of a mass in the lacrimal fossa. Ocular displacement was manifest as 2–12 mm of relative proptosis (40 patients) and inferomedial displacement of the globe (42 patients). A mass was palpable in the lacrimal fossa of 48 patients, extending superonasally in seven and inferotemporally in nine.

Generalised limitation of ocular movement, rarely conforming to a neurological paresis (cases 23, 27, 35), was present in 40/49 patients. Abnormal trigeminal nerve function, generally hypoaesthesia, was recorded in 9/43 patients.

Visual acuity was reduced by two or more Snellen grades with respect to the other eye in 10/47 patients and optic disc swelling or choroidal folds were present in 12/47 cases.

RADIOLOGY

The radiological features of the three principal types of tumour are presented in Table 3. Expansion of the lacrimal fossa was evident on 81% of the CT studies and extraorbital extension or invasion of bone in 36% (Fig 2). Calcification was detectable at soft-tissue CT window settings in 14 (33%) patients and typically had a finely stippled pattern (Fig 2).

Radiologically manifest bone invasion by adenoid cystic carcinoma appears unrelated to the duration of symptoms at ophthalmic presentation ($t=1\cdot29$, $p>0\cdot20$).

PATHOLOGY

Review of the tumour histology resulted in the identification of 38 (76%) adenoid cystic carcinomas (Figs 3A-3D), six (12%) carcinomas which appeared to have developed within

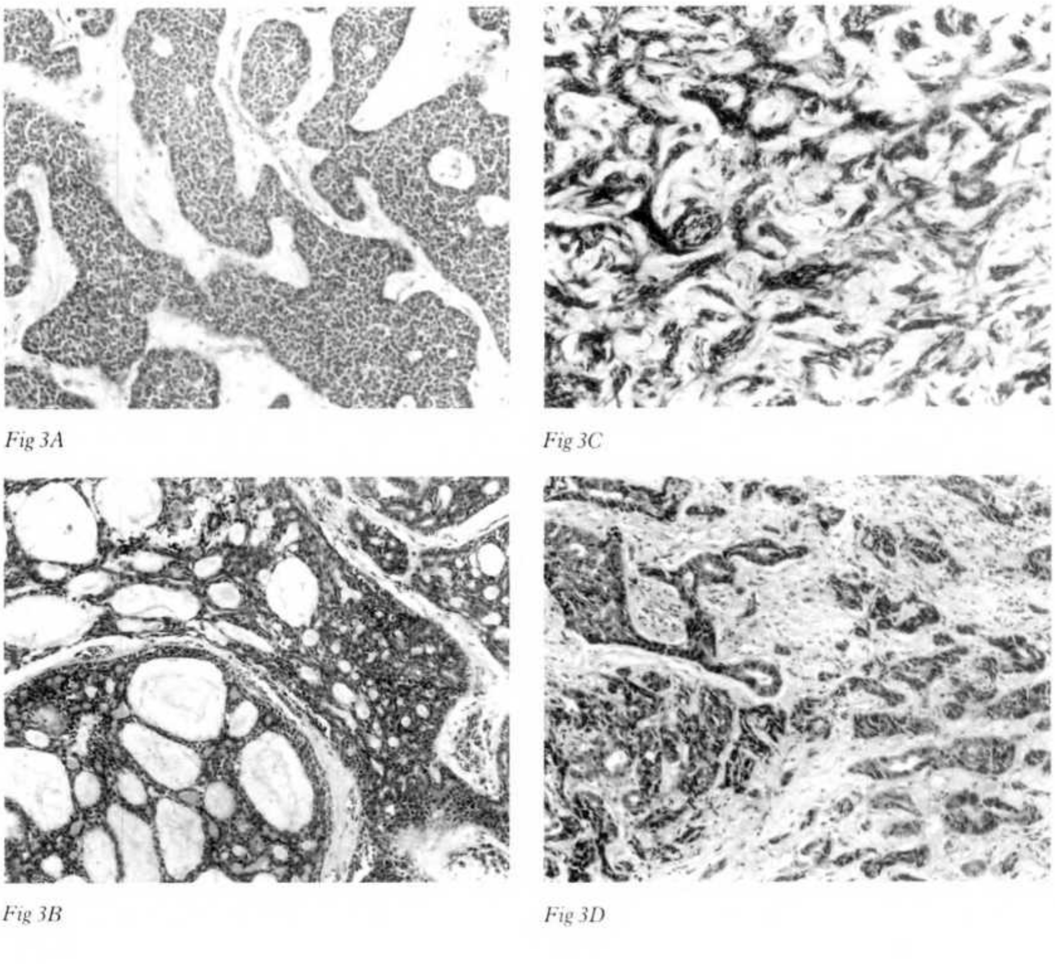

Fig 3A

Fig 3C

Fig 3B

Fig 3D

Figure 3 Adenoid cystic carcinoma: (3A) Basaloid pattern: *Composed of connecting islands of compacted cells, the cells having very little cytoplasm and a superficial resemblance to cutaneous basal cell carcinoma. (3B)* Cribriform pattern: *Consisting of lobules in which the cells are arranged to create multiple cystic spaces of varying size. (3C)* Sclerosing pattern: *In which cords of darkly stained tumour cells are separated by interwoven bands of fibrous tissue. (3D)* Tubular pattern: *Where many of the tumour cells, embedded in fibrous stroma, are forming tubular structures. (Haematoxylin and eosin, ×90.)*

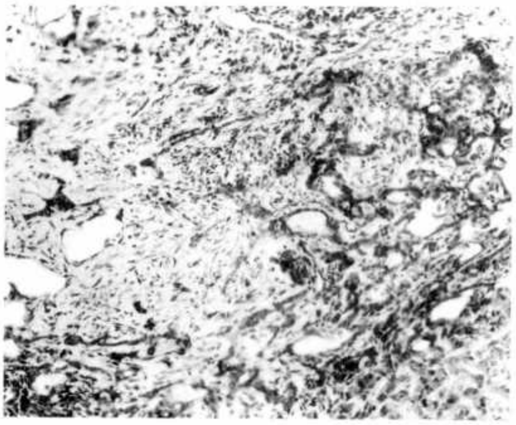

Figure 4 Carcinoma in pleomorphic adenoma (malignant mixed tumour). Infiltrative tumour, giving rise to cords and ductular forms, are superimposed on tumour with a more benign appearance. (Haematoxylin and eosin ×90.)

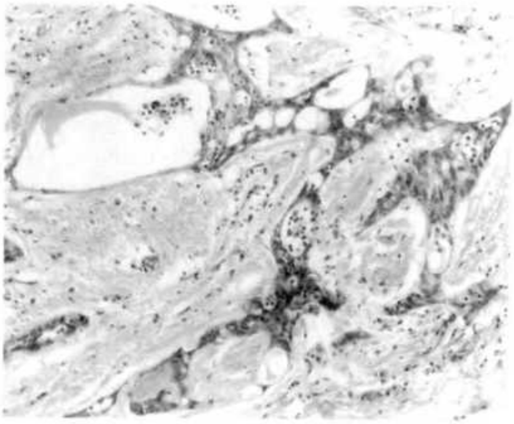

Figure 6 Mucoepidermoid tumour: Irregular cords of epidermoid cells, some including mucus-filled vacuoles, are embedded in a conspicuously mucoid structure (Haematoxylin and eosin, ×90.)

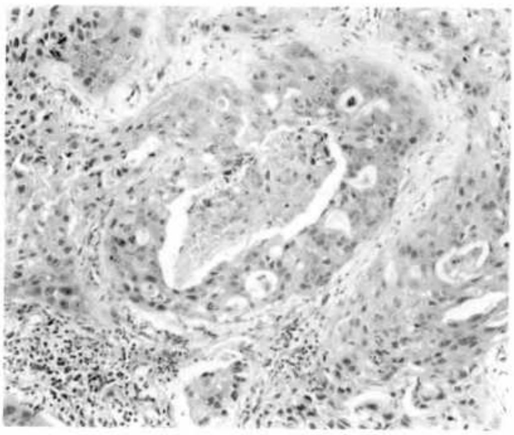

Figure 5 Adenocarcinoma: The tumour cells have abundant eosinophilic cytoplasm and are attempting to form ductules filled with inspissated cell residues and mucus. (Haematoxylin and eosin, ×90.)

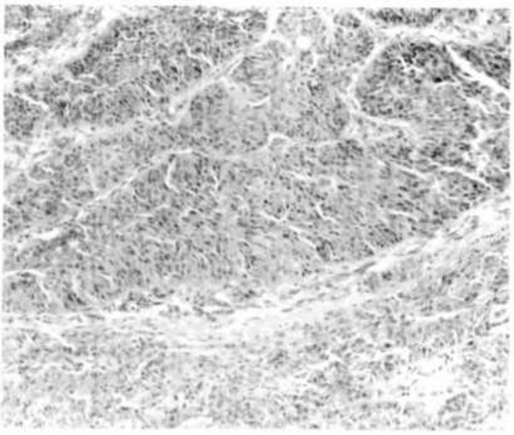

Figure 7 Squamous (epidermoid) carcinoma: Solid trabeculae of squamous epithelium are seen in the absence of mucoid elements. (Haematoxylin and eosin, ×90.)

pleomorphic adenomas (malignant mixed tumours; Fig 4), four (8%) pure adenocarcinomas (Fig 5), one mucoepidermoid tumour (Fig 6), and one squamous carcinoma (Fig 7). Two lesions were reclassified as pleomorphic adenomas (cases 9 and 24; Table 1).

The diagnosis was revised in 12 of the 24 patients reported on previously[15]: eight tumours originally considered poorly differentiated or anaplastic carcinomas were reclassified as basaloid adenoid cystic carcinomas, and two, originally diagnosed as malignant mixed tumours, were reclassified as a mucoepidermoid carcinoma or an adenoid cystic carcinoma. Two patients (one reported previously as malignant mixed tumour, one as adenoid cystic carcinoma) were considered to have pleomorphic adenomas displaying marked, but benign, cellular pleomorphism (cases 9 and 24).

Classification as basaloid adenoid cystic carcinoma depended on finding at least half of the tumour, as seen in biopsy material, to consist of islands, or larger aggregates, of closely packed cells with hyperchromatic nuclei and scanty cytoplasm (13 cases; Fig 3A). Other histological types superimposed upon, or occasionally replacing, the more usual cribriform pattern (27 cases; Fig 3B), comprised sclerosing (eight cases; Fig 3C) or tubular changes (two cases Fig 3D).

TREATMENT AND OUTCOME

The treatment and survival from its beginning are given in Table 1. Tumours recurred in the soft tissues or bones near the site of the primary tumour (26 cases), metastasis to regional lymph nodes or distant sites being less frequent and occurring later in the course of the disease (13 cases).

Mean survival for those who died with active tumour was similar in the three principal types of tumour (Fig 8; $F=0\cdot07$, $p>0\cdot05$). However, the survival time for those who died with active adenoid cystic carcinoma (mean 3·0, SD 3·2 years) was significantly less ($t=3\cdot82$, $p<0\cdot01$) than for those who died without active tumour (mean 11·4, SD 4·8 years), implying palliation, or possibly cure, in some of the latter.

The interval before clinical evidence of tumour recurrence was similar ($t=0\cdot12$, $p>0\cdot90$) in 16 cases of adenoid cystic carcinoma (mean 2·2; range 0·2 to 9·3 years) and four cases of malignant mixed tumour (mean 2·4; range 1·3 to 3·2 years).

Actuarial analysis suggests that the risk of recurrent adenoid cystic carcinoma continues for many years after treatment (Fig 9).

Recurrence of adenoid cystic carcinoma was significantly ($S=0\cdot01$) more frequent in the 13 cases where half or more of the tumour showed basaloid differentiation (9/13 cases; 69%), than in 23 cases in which this pattern was not evident (six recurrences; 26%). This difference is

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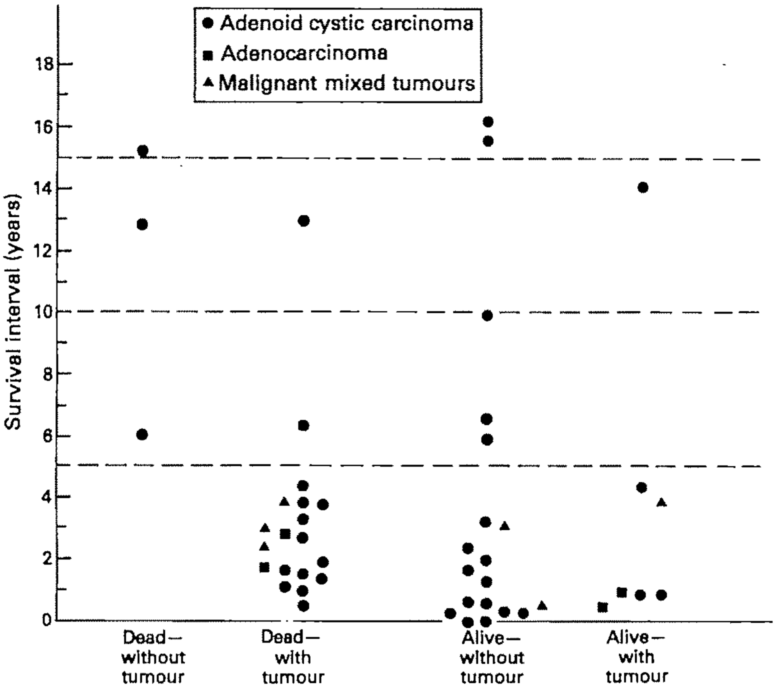

Figure 8 Survival intervals and status after first treatment of 48 patients with lacrimal gland carcinomas. A mass, both clinical and radiological, is often present after treatment. Although such a mass might *contain active tumour cells, such patients are categorised as 'without tumour' until the onset of definitive signs of tumour growth.*

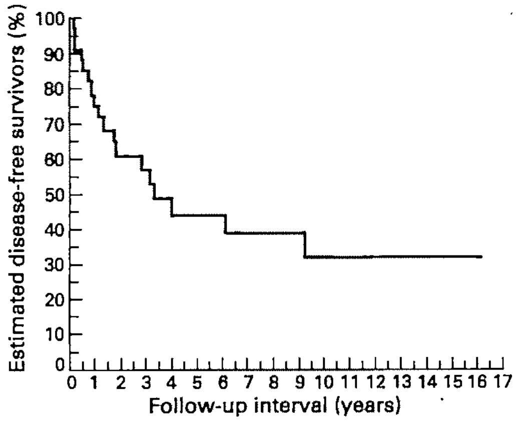

Figure 9 Estimated disease-free survival for patients with adenoid cystic carcinoma after various modes of therapy; Kaplan-Meier analysis based on results from 38 cases.

confirmed with Kaplan-Meier estimates of disease-free survival (Mantel-Haenszel statistic=8·31, $p<0\cdot01$), although most basaloid tumours recurred within 2 years of treatment (Fig 10).

Cranio-orbital resection does not reduce the estimated rate of recurrence of adenoid cystic carcinoma (Fig 11; Mantel-Haenszel statistic=0·86, $p>0\cdot05$).

Discussion

Adenoid cystic carcinoma is, in our experience, the most common type of lacrimal gland cancer and tends to present at an earlier age than other malignant tumours at this site, having a peak incidence in the fourth decade (Fig 1). A second peak, in the sixth decade, might reflect, in the present series, a greater tendency for basaloid tumours to present after the age of 40; Milam and Heath,[17] however, report basaloid tumours to occur at an earlier age than other types.

In contrast to pleomorphic adenomas, malignant lacrimal gland tumours typically present with a short history,[22 24] principally of pain, ocular displacement and a swelling of, or lump in, the upper lid (Table 2). Some patients with malignant mixed tumours may, however, give a long history of ocular displacement or previous surgery prior to a recent progression of symptoms. In the present series 81% of patients had symptoms for less than 1 year at first ophthalmic consultation. Delayed referral to the Orbital Clinic resulted in, on average, more than 4 months' (and up to 33 months') delay before biopsy was performed – which might adversely influence the survival of the patient.

PAIN AS A SYMPTOM WITH LACRIMAL GLAND MALIGNANCY

Pain is an important symptom of lacrimal gland malignancy[22 24] and might be thought to indicate perineural infiltration with a more advanced stage of malignant disease.[19]

Thus, pain occurs more frequently (S=0·02), and earlier, with adenoid cystic carcinoma than with adenocarcinoma or malignant mixed tumours. However, the occurrence of pain with

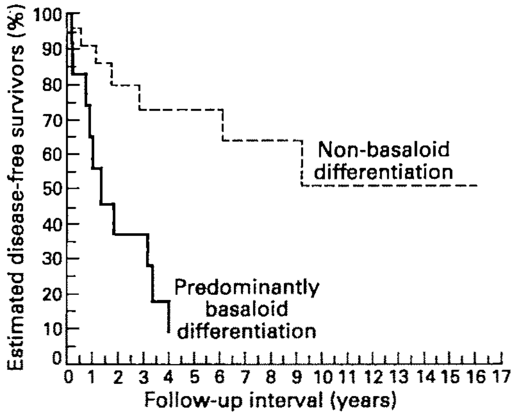

Figure 10 Kaplan-Meier estimated disease-free survival for patients with adenoid cystic carcinoma where histology is either predominantly basaloid (13 cases) or non-basaloid (23 cases). Two cases demonstrated a minority of basaloid differentiation and, hence, could not be included in either group.

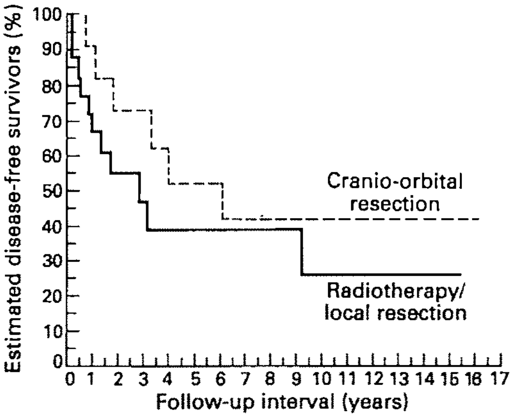

Figure 11 Kaplan-Meier estimated disease-free survival for patients with adenoid cystic carcinoma after extended orbital resection (11 cases) or limited localised therapy (radiotherapy and/or local resection; 27 cases).

Table 4 Survival and clinical recurrence of adenoid cystic carcinoma in 35 patients; two other patients had local resection only and one other had radiotherapy with chemotherapy

	Radiotherapy alone (14)	*Local resection and radiotherapy (10)*	*Cranio-orbital resection, with or without radiotherapy (11)*
Deaths	8 (57%)	2 (20%)	5 (46%)
Mean survival to date (years)	1·7 (0·1–58)	4·8 (0·3–15·4)	7·7 (0·8–16·1)
Interval between treatment and recurrence of tumour (years)	0·79 (0·2–1·8) (8 patients)	6·2 (3·1–9·3) (2 patients)	2·9 (0·8–6·1) (6 patients)

adenoid cystic carcinoma is unrelated to a predominance of basaloid differentiation (S=0·70) or to the incidence (S=0·56) or time (t=0·82, p>0·30) of recurrence. Similarly, pain is unrelated to survival intervals for *all* patients (t=1·86, p>0·05), or for those *without recurrent disease* (t=1·44, p>0·10). The overall duration of symptoms at ophthalmic presentation of adenoid cystic carcinoma was similar in patients with or without tumour recurrence (t=0·72 p>0·40), in those undergoing radiotherapy and/or local, rather than cranio-orbital, resection (t=0·78, p>0·40), in those with or without pain (t=1·46, p>0·10), and in those with or without invasion of bone (t=1·30, p>0·20).

Impairment of trigeminal nerve function might indicate perineural infiltration by tumour. The duration of symptoms was, however, similar in nine patients with and 33 without trigeminal sensory change (t=0·04, p>0·90), and the incidence of pain was similar in the groups S=0·30).

Expansion of the lacrimal fossa occurred in most patients with malignant tumours, and there was frequently radiographic evidence of bone invasion (Table 3), especially on high resolution CT. As reported by Jakobiec and others,[25] lacrimal gland malignancies generally assume a more elongated, rather ill-defined mass, extending along the lateral orbital wall (Fig 2).

CLINICAL OUTCOME IN RELATION TO TUMOUR HISTOPATHOLOGY

Preoperative symptoms or signs do not provide a reliable guide to the quality or duration of relief for patients with lacrimal gland malignancy. Clear signs of extraorbital tumour invasion, such as bone destruction, inevitably increase the risk of tumour recurrence and influence management.

Tumour histopathology is the most significant factor in the survival of patients in the present series. Although patients with recurrent adenoid cystic carcinoma, adenocarcinoma, or malignant mixed tumour have similar intervals, there is considerable variation among patients with adenoid cystic carcinoma.

A preponderance of basaloid differentiation with an adenoid cystic carcinoma is associated with a highly significant (p<0·01) reduction in estimated disease-free survival (Fig 10). This difference is not due to therapeutic bias, the proportion of patients undergoing radiotherapy and/or local, rather than cranio-orbital resection, being similar (S=0·87) in the basaloid and the non-basaloid groups. Likewise, the proportion of patients with pain (S=0·70) and the duration of symptoms at ophthalmic presentation (t=0·39, p>0·60), or at biopsy (t=0·46, p>0·60), were similar.

Without the more usual cystic morphology the solid masses of cells characteristic of basaloid adenoid cystic carcinomas might signify a less differentiated form and be consistent with a worse prognosis. A similar association has been reported with salivary gland tumours,[25] though other variables (such as different anatomical sites of the glands) complicate interpretation of results. Other authors have not convincingly demonstrated these findings with salivary gland tumours.[21 27] For adenoid cystic carcinoma of the lacrimal gland Gamel and Font[16] report a markedly reduced survival with basaloid differentiation (median survivals 3 and 8 years, respectively). Lee *et al*,[18] while failing to confirm this, suggested that the well differentiated cribriform pattern of adenoid cystic carcinoma carries a better than average prognosis.

A tubular pattern for adenoid cystic carcinoma of the salivary glands is associated with improved prognosis,[26] though this has not been shown with such tumours of the lacrimal gland.[18] As in the series of Gamel and Font,[16] the prevalence of tumours with tubular differentiation in the present series is inadequate for such analysis.

CLINICAL OUTCOME IN RELATION TO THERAPY

Because of the small numbers, the influence of therapy on the outcome of adenocarcinoma or malignant mixed tumours is difficult to assess in the present series. However, these carcinomas appear to have a high recurrence rate within 2 to 3 years of treatment, and survival is poor (Fig 8).

Adenoid cystic carcinomas continue to recur for many years after initial treatment (Fig 9).[1 7 11-15 18 19] With current follow-up intervals the rate of disease-free survival is not improved (p>0·05) by cranio-orbital resection in those patients with clinically and radiologically localised tumours. However, the relatively high proportion of cranio-orbital resections among patients surviving for more than 10 years (Table 1) suggests that this treatment might lead to improved survival; this will be evident only with prolonged follow-up of the present cohort (for example, after another 10 years).

Twenty seven patients were regarded, on clinical and/or radiological grounds, as having adenoid cystic carcinoma extending outside the orbit or widely infiltrating orbital structures and, therefore, unsuited for cranio-orbital resection. This group received radiotherapy (25 cases) and/or local resection of tumour (12 cases).

As compared with radiotherapy alone, local resection with radiotherapy for adenoid cystic carcinoma delayed significantly (t=3·89, p<0·01) the recurrence of tumour and was associated with a slightly longer survival (t=1·93, 0·1>p>0·05; Table 4). Although debulking of tumours might enhance the efficacy of radiotherapy, interpretation of the present results is difficult because of the small numbers and clinical selection of patients for treatment. However, it remains uncertain whether radiotherapy[14] affects the outcome of these malignant tumours.

Eleven patients, considered preoperatively to be 'curable', underwent cranio-orbital resection (generally with radiotherapy) for adenoid cystic carcinoma; at surgery, however, tumour was seen to have invaded bone in two patients and was therefore possibly incurable. These 11 patients undergoing cranio-orbital resection may be compared with 10 having local resection with radiotherapy (considered beyond surgical 'cure'): tumour recurred in 6/11 patients (or 4/9, excluding those with known bone invasion) after cranio-orbital resection, as compared with 2/10 after debulking and radiotherapy. Perhaps because of the small numbers, these proportions are similar (S=0·12), but so too are the survival intervals in the two groups (Table 4; $t=1{\cdot}17$, $p>0{\cdot}2$). Clinically apparent tumour recurrence was later after debulking and radiotherapy, but this did not reach significance (Table 4).

It is possible, therefore, that local resection of tumour with radiotherapy might be as effective as cranio-orbital resection wth radiotherapy in preventing local recurrence of malignant lacrimal gland tumours: larger numbers of patients with longer follow-up will resolve this point. Moreover, for tumours with very poor prognosis, namely adenocarcinomas and basaloid adenoid cystic carcinomas, there might be a strong case for the use of radical external beam radiotherapy or implant radiotherapy. A protocol for the future treatment of lacrimal gland carcinoma is being developed.

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Dermofat grafts to the extraconal orbital space

G E Rose, R Collin

Abstract

Dermofat grafts were placed in the upper or lower lid sulci in 35 subjects, aged from 11 to 59 years, to improve the cosmesis of volume deficient sockets or prevent tissue adhesion. Volume enhancement and cosmetic improvement were achieved in 31 patients, in whom useful vision was present in 13/22 after previous trauma, in 4/4 with facial clefting, and in 3/3 with orbital or facial fat atrophy. Grafts were used successfully in nine patients to prevent scar formation after division of adhesions between the eyelids or levator muscle and the orbital margins. A reduction of the bulk of upper-lid grafts was required in three cases; histopathology of the excised fat showed relatively minor degrees of inflammation, atrophy, and fibrosis.

Since the introduction of dermofat grafting[1] the most common ophthalmic use has been the augmentation of soft tissues in volume deficient sockets after enucleation; such grafts are placed within the residual tissues of the intraconal space.[2-8] Unlike non-autogenous materials used for volume augmentation dermofat grafts (being autogenous) have negligible risk of rejection, although there is some tendency for lipolysis and resorption of adipose tissue in the early post-operative period.

Free dermofat may be grafted into the extraconal spaces,[4, 8-11] where the dermis is apposed to the periosteum of the orbital rim or to other suitably vascularised structures; in this situation the grafts can provide both volume augmentation and a valuable barrier to the formation of scar tissue.

The results of dermofat grafting to the extraconal orbital space in 35 patients are reviewed in this paper.

Patients and methods

Patients having extraconal dermofat grafting between 1980 and 1991 were identified from the surgical records at Moorfields Eye Hospital.

All surgery was performed under general anaesthesia. The dermofat graft was taken from the buttock, at a point half-way between the ischial tuberosity and ipsilateral greater trochanter, using well-established techniques.[12] Parenteral antibiotics were given at the time of harvesting the tissue.

Moorfields Eye Hospital, London
G E Rose
R Collin

Correspondence to:
Mr G E Rose, MS, FRCS,
Moorfields Eye Hospital, City
Road, London EC1V 2PD.

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EXTRACONAL DERMOFAT GRAFTS FOR VOLUME DEFICIENT SOCKETS

For volume deficient sockets with deep upper-lid sulci the orbital rim was approached through a skin crease incision at a level determined preoperatively. The orbital septum was divided and the fat along the anterior part of the orbital roof displaced inferiorly. The dermal surface of the dermofat graft was attached to the periosteum of the orbital roof using 6/0 polyglycolate sutures, with care being taken to avoid damage to the supraorbital neurovascular bundle and the trochlea. The skin crease was reformed by sutures attached to the anterior part of levator muscle or aponeurosis.

A subciliary blepharoplasty approach was used for dermofat grafting to the interior orbital rim. The rim was approached in a similar fashion to the upper lid and the graft secured to the anterior periosteum of the orbital floor.

EXTRACONAL DERMOFAT GRAFTS FOR PREVENTION OF TISSUE ADHERENCE

Areas of adhesion between lid tissues and orbital rim were approached as in the volume deficient sockets. Tissue adhesions were separated with respect for natural tissue planes, and any necessary repair of tissues (such as levator muscle) performed. The dermofat graft was then sutured to the periosteum overlying the site of tissue adherence and the superficial layers closed.

In some cases other measures, such as medial canthoplasty or lateral tarsorrhaphy, were used

Table 1 Extraconal dermofat grafts in 35 patients

Reason for dermofat grafting	*Status of the eye*	*Initial ocular/orbital pathology*
26 cases for cosmetic improvement	Sighted* eyes (13)	9 post-traumatic 1 post-operative 1 hemifacial atrophy 1 blepharochalasis 1 maxillary dysplasia
	Blind eyes (2) or enucleated (11)	8 post-traumatic 5 tumours
4 cases for tissue separation	Sighted* eyes (4)	1 post-traumatic 3 facial clefts
5 cases for tissue separation and cosmetic improvement	Sighted* eyes (3)	3 post-traumatic
	Blind eyes (1) or enucleated (1)	1 post-traumatic 1 post-irradiation

*'Sighted' denotes an acuity of finger-counting or better.

Table 2 Ocular characteristics for patients undergoing dermofat grafting to the extraconal orbital space

Ocular characteristics	*Mean value*	*Number of cases*
Depth of upper lid sulcus (graded 0–3)		
preoperative	2·4	29
postoperative	0·7	29
Depth of lower lid sulcus (graded 0–3)		
preoperative	1·1	10
postoperative	0·3	10
Degree of enophthalmos (graded 0–3)		
preoperative	1·3	35
postoperative	1·3	35
Palpebral aperture (affected side; mm)		
preoperative	8·9 (9·0)*	34 (33)*
postoperative	7·8	30
Levator function (affected side; mm)		
preoperative	9·0 (14·4)*	31 (31)*
postoperative	8·2	29

* Values for the unaffected (healthy) side, where appropriate.

to help eyelid closure and the eyelid was placed on traction if needed.

Ocular characteristics were graded from the case notes and, where possible, from clinical photographs. Subjective measures were arbitrarily graded as '0', '1' (mild), '2' (moderate), or '3' (marked) and other measurements, such as palpebral aperture or levator function, were assessed using standard methods.

Results

Thirty five patients (15 male, 20 female), aged from 11 to 59 years, underwent extraconal dermofat grafting to the right, left, or both sockets (20, 13, and two cases, respectively); grafts were placed in the upper eyelid (29 cases) or lower eyelid sulcus (10 cases). Follow-up intervals were from 4 months to 7 years (median 1 year).

Sulcus dermofat grafts were performed in 22 sockets after previous trauma; enucleation has been performed in six cases and 13/16 remaining had sighted eyes (Table 1). After prior ocular surgery or tumour six patients had grafts solely for volume augmentation (five enucleated) whereas one enucleated socket was grafted also to prevent tissue adhesions (Table 1). Three

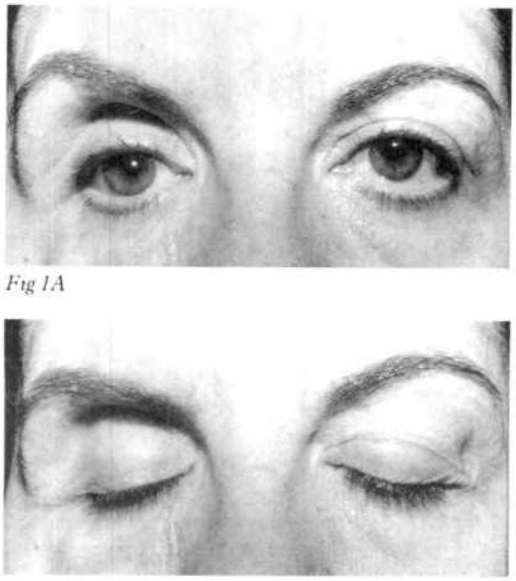
Fig 1A

Fig 1B

Figure 1 A deficiency of soft tissue volume is present in the right orbit after blowout fracture; although the upper lid sulcus is very deep, the vision, ocular movements, and eyelid movements are all normal (A, B).

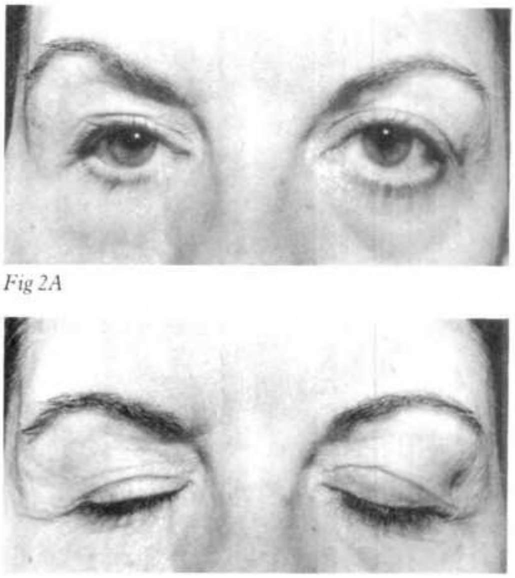
Fig 2A

Fig 2B

Figure 2 The same patient as Figure 1, 3 months after dermofat grafting to the upper eyelid sulcus; eyes (A) open, (B) closed.

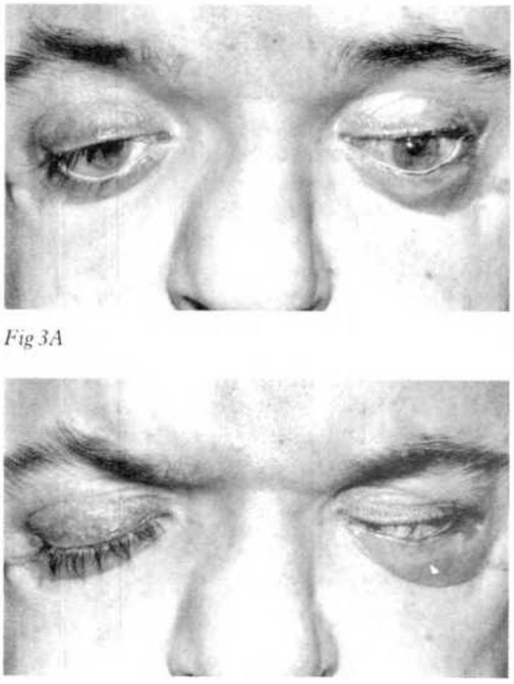
Fig 3A

Fig 3B

Figure 3 Patient with bilateral lower lid retraction (A), lagophthalmos (B) and secondary corneal exposure; there is adherence of malar scars to the maxillae, which were advanced during surgery for Apert's syndrome.

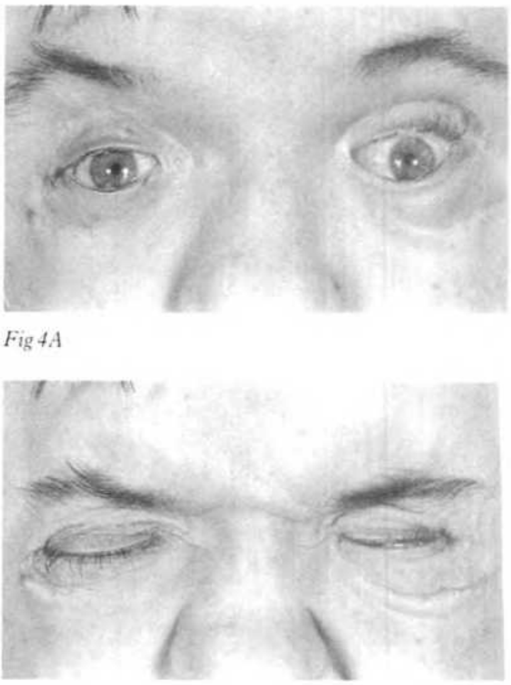
Fig 4A

Fig 4B

Figure 4 Same patient as Figure 3, 6 months after freeing of tissue adhesions and dermofat grafting on the inferior orbital rims; the grafts reduce adherence of scar tissues and improve the malar profile. Eyes (A) open, (B) closed.

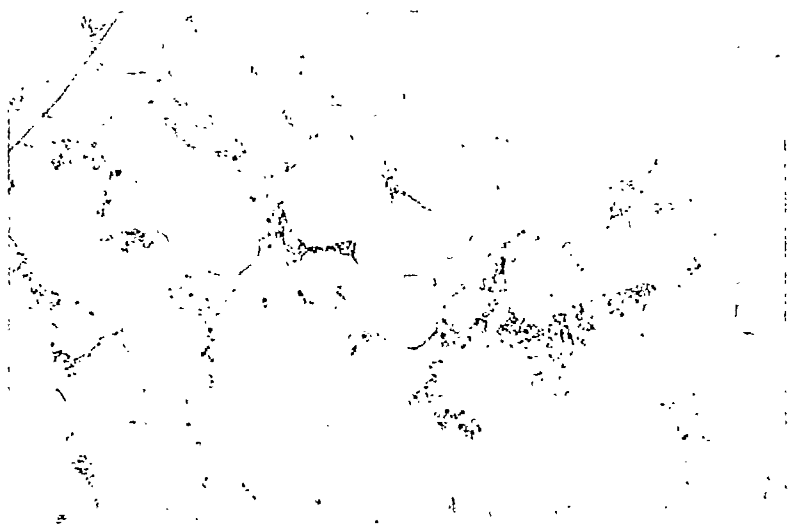

Figure 5 Specimen of dermofat removed 1 year after grafting into the upper lid sulcus. There is no significant inflammation or fibrosis and the histological structure of the fat is well maintained.

patients had severe orbital fat atrophy (one blepharochalasis syndrome, one hemifacial atrophy, and one maxillary hypoplasia) and were grafted for volume deficient sockets, whereas three other patients with facial clefts had grafts to prevent tissue adherence between the eyelids and orbital margins; all six of these patients had sighted eyes. Dermofat grafting was combined with other procedures to the upper eyelid in eight patients – namely, levator muscle resection (six cases), recession of the upper-lid retractors (one case), and entropion repair (one case).

There were no intraoperative complications even in cases where the orbital roof had been damaged at initial injury. As a result of surgery, however, two patients developed a transient supraorbital neuropraxia. Lipolysis occurred in many grafts and a minimal discharge of liquefied fat from the skin incision was quite common. One patient developed a staphylococcal abscess at 2 months after surgery, which resolved with drainage and systemic antibiotics; there was however, marked reabsorption of fat from the graft and subsquent adhesion between the eyelid and the orbital margin.

Despite reabsorption of fat. there was good augmentation of tissue volume and reformation of the upper-lid skin crease with all grafts (Table 2; Figs 1 and 2). Similarly where the graft was used as a 'spacer' there was a satisfactory prevention of tissue adhesion which improved eyelid movements (Figs 3 and 4); in the overall group of 35 patients, however, there was a tendency to a slight reduction of palpebral aperture (due to gravitational ptosis) but almost no change in the elevator function (Table 2).

Five patients had anterior approach ptosis surgery as a secondary procedure (three planned before dermofat grafting). The bulk of the graft was too great in four patients and three had a secondary debulking (one with ptosis correction, one with skin crease reformation).

Discussion

Free dermofat grafts into the extraconal space are useful for augmenting the tissues within volume deficient sockets. The grafts, placed in the upper and/or lower sulci, are particularly useful where volume has already been augmented by intraconal[8] and orbital floor implants,[8 13] or where there is a sighted eye and orbital floor implantation would carry a significant risk of optic nerve damage.

Volume enhancement was the prime indication for dermofat grafting in 26/35 patients in this series, of whom one half had usefully sighted eyes (Table 1; Figs 1 and 2); enhancement of socket volume had been performed where possible by intraconal and orbital floor implantation. There were five patients in whom volume enhancement was a secondary gain after the prime necessity for a 'spacer' to prevent tissue adhesions; three of these five patients had sighted eyes (Table 1; Figs 3 and 4). The aim in all patients was to slightly overcorrect volume deficiency, to allow for postoperative fat reabsorption, and loss of tissue volume was not a significant problem (Figs 2 and 4). All 31 patients had a satisfactory reduction in the depth of the eyelid sulci, although the grafts remained too bulky in four patients and were surgically reduced in three cases.

The anterior approach to grafting the upper lid allows both reformation of the skin crease and also surgery to correct ptosis or upper-lid entropion. A postoperative ptosis is common after upper-lid grafting but typically improves as reabsorption of the fat and tissue oedema occurs; secondary ptosis surgery was needed in 5/31 patients and had been expected in three of these five cases.

Mobile tissues, such as the eyelids or levator muscle, may become adherent to the fixed orbital margins after trauma; after surgical separation a re-adherence of these tissues is almost inevitable unless the two surfaces are kept apart by the use of a 'spacer' – such as dermofat or silicone sheet. A dermofat graft is less liable to extrusion or infection than non-autogenous materials, the risk of infection being particularly great in post-traumatic cases where sinus fractures are fairly common. Unlike silicone sheet, which tends to be prominent when placed near the orbital margin, dermofat grafts have a smoother contour when straddling the orbital rim – a position often required for 'spacer' grafts (Fig 4).

The primary indication for dermofat grafting in nine patients was to provide a 'spacer' and in eight of these the scars were either post-traumatic or postoperative (Fig 3). Eye closure was improved in all cases and the position and movements of the upper (six cases) or lower (three cases) eyelids were better. In some of this group the visual acuity improved after surgery as the preoperative exposure keratopathy resolved.

With the exception of one patient, in whom the graft became infected, the degree of post-operative fat reabsorption was not sufficent to impair the surgical results. Where the dermofat graft required debulking it is of particular interest that the excised tissue from two cases showed only minimal inflammatory cell infiltrates or fibrosis and that the cellular architecture for the adipose tissue was very well maintained (Fig 5); this finding of almost normal fat architecture is similar to that in another report.[14]

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Non-traumatic acquisition of herpes simplex virus infection through the eye

S B Kaye, C Shimeld, E Grinfeld, N J Maitland, T J Hill, D L Easty

Medical Sciences Building, University Walk, Bristol BS8 1TD

Department of Ophthalmology
S B Kaye
C Shimeld
E Grinfeld
D L Easty

Department of Pathology and Microbiology
N J Maitland
T J Hill

Correspondence to:
S B Kaye.

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Abstract

Primary ocular herpes is usually seen as a follicular conjunctivitis and blepharitis, with or without involvement of the cornea. It is unknown, however, to what extent asymptomatic and/or subclinical primary disease occurs, and whether primary ocular herpes follows direct droplet spread to the eye. Previous models of murine ocular herpes have used trauma (scarification) to introduce virus into the cornea, producing disease which results in significant corneal scarring. To mimic a likely route of infection in humans, a droplet containing virus was placed on the mouse eye and clinical disease recorded. At least 1 month after inoculation, serum was assayed for neutralising antibodies and the cornea, iris, and trigeminal ganglion were investigated for evidence of herpes simplex virus type 1, by cocultivation and the polymerase chain reaction. Some animals showed a severe ulcerative blepharitis with little to no involvement of the cornea, while disease was undetectable in others. The development of disease depended on the dose and strain of virus and age of the animal, with older mice appearing more resistant. Virus was isolated from the trigeminal ganglion of younger animals inoculated with higher doses of virus, after 21 days in culture, suggesting that latency had been established. Neutralising antibodies were present in most mice irrespective of the presence of recognisable clinical disease. Using primers for the thymidine kinase and glycoprotein C regions of the viral genome, herpes simplex virus type 1 DNA was found in the cornea, iris, and trigeminal ganglion of most animals and showed a good correlation with the presence of neutralising antibodies. It would thus appear that herpes simplex virus type 1 is able to accede into the cornea, iris, and trigeminal ganglion following non-traumatic application of virus onto the mouse eye. This model mimics primary ocular disease in humans and may be useful for studies on recurrent disease and the spread of ocular herpes.

Herpes simplex virus type 1 (HSV-1) is an important cause of ocular disease in both developed and underdeveloped countries.[1,2] At present, despite advances in elucidating the molecular biology of HSV-1,[3] we are still unable to prevent infection and the subsequent recurrences which may occur in the eye. The mechanism by which virus gains access to the cornea and trigeminal ganglion (TG) to infect and to establish a latent infection is central to the pathogenesis of herpes keratitis (HSK), its prevention, treatment, and eventual eradication.

The frequent occurrence of asymptomatic salivary shedding of HSV-1,[4,5,6] provides indirect evidence of asymptomatic primary oral disease and for implicating the mouth as the main site for the acquisition and spread of HSV-1 within the community. The proportion of cases of primary herpetic eye disease that are asymptomatic however, is not known and they may play an important role in the development of recurrent corneal disease. The sensitive polymerase chain reaction (PCR), has been used to detect HSV-1 DNA in the corneas of patients with previous HSK,[7,8,9] in the absence of an isolation of cocultivation.[10] HSV-1 DNA has also been detected in non-herpetic corneal diseases such as aphakic bullous keratopathy, keratoconus, and interstitial keratitis,[7,8,10] a finding consistent with the occurrence of asymptomatic HSV-1 ocular infection.

Primary ocular infection in the mouse has been well studied[11-14] using corneal trauma for the inoculation of virus. Although non-invasive inoculation has been used in rabbits[15] there has been only one previous murine study in which disease was reported.[12] The clinical findings, however, were not described nor were the ocular tissues and TG tested for the isolation of virus.

After scarification the disease which occurs is predominantly corneal, and usually results in extensive scarring. In humans, however, there is no evidence that such corneal trauma is necessary for ocular infection or the establishment of latency. In addition, cases of primary ocular herpes in humans with follicular conjunctivitis show varying degrees of blepharitis with or without corneal involvement and generally heals with no residual damage.[16,17]

In this study, virus was applied to the mouse eye in droplet form, in the absence of trauma, to mimic a possible route of human primary ocular infection. Following inoculation mice were examined for clinical disease and the humoral immune response assessed for serum neutralising antibodies. At least 1 month after infection, the cornea, iris, and trigeminal ganglion were investigated for evidence of HSV-1 by the PCR or for a latent infection by cocultivation.

Methods

INOCULATION

NIH/OLA inbred female mice were anaesthetised with 0·1 ml of Hypnovel (Roche) and 0·1 ml of Hypnorn (Janssen). The left eye was inoculated either by application of virus onto the cornea in a 5 µl or 10 µl droplet (topical inoculation) of medium (medium E-199, 0·15% sodium bicarbonate, streptomycin 0·1 mg/ml, polymyxin B sulphate 50 U/ml penicillin 10^2 U/ml (SPA) and 20% fetal calf serum) or by scarification through the 5 μl or 10 μl droplet of medium containing virus.[12] The lids were not massaged and the animals were placed on shredded paper with their left eye uppermost. Control mice were inoculated with uninfected Vero cells prepared in the same manner as the virus pool (mock inoculum).

EXPERIMENT 1

Fifty-six 12·5-week-old mice were divided into four groups and inoculated with 5 μl as follows: group A (n=30), topically with 5×10^5 pfu of HSV-1 strain McKrae,[18] group B (n=5) topically with mock inoculum, group C (n=14) by scarification with 5×10^3 pfu of HSV-1 strain McKrae and group D (n=5), by scarification with mock inoculum.

Two mice were left uninfected for additional PCR controls.

EXPERIMENT 2

Fifty, 5·5-week-old mice were divided into four groups and inoculated with 10 μl (groups E to G) or 5 μl (group H) as follows: group E (n=15) and group G (n=15) topically with 10^6 pfu of HSV-1 strain McKrae or strain SC16[19] respectively, and group F (n=10) and group H (n=10), by scarification with 10^4 pfu of HSV-1 strain McKrae or SC16 respectively.

EXPERIMENT 3

Twenty-four 4-week-old mice were topically inoculated with 5 μl of medium containing 3×10^5 pfu of HSV-1 strain SC16 and four animals were left uninfected.

CLINICAL EXAMINATION

Both eyes were examined for signs of disease using slit-lamp biomicroscopy, daily for the first week, alternate days for the second week, and thrice weekly for the remainder of the experiments.

DISSECTION AND REMOVAL OF TISSUES

Mice were killed at 8 weeks (expt 1) and 4 weeks (expt 2) post infection, by an overdose with pentobarbitone (Sagatal, May & Baker). A 2 mm diameter corneal disc was trephined from the left cornea, following which a substantial piece of iris and ciliary body was removed by microdissection. The left trigeminal ganglion was then removed. Tissues from animals in expt 1 were either cocultivated with Vero cells (with and without 15 μM 5'azacytidine) for the isolation of virus, or immediatly frozen and kept at −70°C for subsequent DNA extraction. Tissues from all animals in expt 2 were cocultivated.

TITRATION OF SERA FOR ANTIBODIES TO HSV-1

Serum collected before inoculation, at 8 weeks (expt 1) and 4 weeks (expt 2) after infection was titrated for neutralising antibodies using the plaque reduction method.[20] The ED_{50} was calculated by probit analysis. Neutralising antibodies were not measured in experiment 3.

POLYMERASE CHAIN REACTION

The cornea, iris, and TC (expt 1) were digested with proteinase K (Sigma) and the DNA extracted as previously described.[10] Dilutions were made so that 5 μl of nucleic acid solution contained 150–250 ng of DNA. Negative control DNAs from the corneas, irides, and TGs of mock infected and uninfected mice were prepared in the same manner. Positive control DNA was obtained from Vero cells acutely infected with HSV-1.

Three sets of primers were used in the PCR: two for overlapping regions of the thymidine kinase gene (TK), and one pair for the glycoprotein C (gC) region. The two TK primers, TK1[21] and TK2 amplify a 110 and a 278 bp fragment respectively, and the gC[10] a 115 bp fragment. The TK1 primers were used as they have been previously shown to be informative in mouse tissue[21] and were thus used as a control. The TK2 primers have the sequences

5'-TTATTGCCGTCATAGCGCGG
5'-GGCGACCTGTATAACGTGTT

and were selected for optimisation using Primer Designer program (Scientific and Educational Software, Pennsylvannia, USA). A GeneAmp DNA amplification kit (AmpliTaq, Perkin Elmer) was used for the PCR, the 50 μl reaction mixture containing 5 μl of total nucleic acid mixture. Following 60 cycles of denaturation (94°C), annealing (55°C), and extension (72°C), on an automated thermal cycler (Perkin Elmer), products were separated by polyacrylamide or agarose gel electrophoresis and visualised by staining with ethidium bromide. A pBR322 DNA-Hae 111 digest (Sigma) was used as a molecular size standard. In all experiments a (no-DNA) blank control was included. A titration of HSV-1 infected Vero cell DNA from 1 μg to 10^{-4} pg, showed the TK2 and gC primers to give better signals than the TK1 primers (confirming the TK2 optimisation).

PRODUCT DIGESTION AND HYBRIDISATION

The identity of the product was confirmed by restriction endonuclease digestion using Sma 1 (Boehringer Mannheim) for TK1 (to produce 73 and 37 bp fragments) and TK2 (210 and 68 bp fragments), and Ksp 1 (Gibco BDH for the gC products (to produce 59 to 56 bp fragments) as previously described.[10] Additional confirmation of the TK products was by Southern blot hydbridisation. A 5'end labelled probe from a 26 base oligonucleotide[10] corresponding to a sequence between the TK1 and TK2 primers was prepared using [γ-^{32}P]ATP (Amersham) and 20 U of polynucleotide kinase (Pharmacia). Hybridisation was in 5 ml/200 cm^2 of rapid prehybridisation buffer (Amersham) at 65°C for 1·5 hours, followed by two 10-minute washes at 20°C in2×SSC, and one 15 minute wash at 65°C in 1×SSC and 0·1% SDS.

Table 1 *Disease, antibody response, cocultivation, and PCR results*

Group (n)	*Inoculation*	*Isolations**	*ED_{50}†*	*PCR‡*	*Disease*
Expt 1 (12½ week-old mice)					
A (30)	5×10^{5} pfu McK 5 µl topical	0/14	14	11/16 C 7/16 I 8/16 TG	None
B (5)	mock inoculum 5 µl topical	0/2	0	0/3 All	None
C (14)	5×10^{5} pfu McK 5 µl scarified	5/6 TG (12) 0/6 I 0/6 C	157	5/5 C 4/5 I 5/5 TG	13/14 severe lid and corneal 3 encephalitis
D (5)	mock inoculum 5 µl scarified	0/2	0	0/3 All	None
Expt 2 (5½-week-old mice)					
E (15)	10^{6} pfu McK 10 µl topical	3/15 TG (21) 1/15 I (23) 0/15 C	116	–	10/15 mild blepharitis
F (10)	10^{4} pfu McK 5 µl scarified	7/9 TG (14) 0/9 I 0/9 C	880	–	10/10 severe lid and corneal
G (15)	10^{6} pfu SC16 10 µl topical	5/14 TG (15) 0/14 I 0/14 C	237	–	13/15 mild lid 9/15 mod lid 1 encephalitis
H (10)	10^{4} pfu SC16 5 µl scarified	6/8 TG (15) 2/8 I (22) 0/8 C	330	–	10/10 severe lid and corneal 2 encephalitis

*Number of isolations/number tested (average no of days in culture to isolate virus following removal of tissue)
†Mean neutralising antibody response
‡Number of specimens positive with three primers/number tested
McK=HSV-1 strain McKrae; SC16=HSV-1 strain SC16; C=cornea; I=iris; TG=trigeminal ganglion

REACTIVATION OF LATENT INFECTION

On day 30 after inoculation, mice were irradiated with UV light (Hanovia lamp 420 nm, 4·02 J/cm^2) for 2 minutes with the left eye proptosed.[22] Before irradiation (days −4, −3, 0) and after irradiation (days 1, 2, 3, 4) eye washings were taken for the isolation of HSV-1.[12]

Results

CLINICAL RESPONSE (TABLE 1)

Experiment 1

No evidence of detectable disease could be found in group A (topical virus inoculation) or in group B (topical mock inoculum).

All scarification lines in groups C and D had healed by day 2. From day 3, 13/14 mice from group C developed disease. This began as blepharospasm, followed by the development of lid ulceration, central corneal geographic ulceration, and varying degrees of mydriasis. By the end of the third week there was lid scarring with hair loss and the corneas were vascularised and scarred.

The right eyes remained normal throughout the study.

Experiment 2

Topically infected mice (groups E with strain McKrae and G with strain SC16) developed disease in the inoculated eye in the following manner: mild lid oedema and a squamous blepharitis was present by day 3 (10/15 in group E and 13/15 in group G), with two mice in Group G showed lid vesicles. By the end of the first week mice had developed varying degrees of the upper an lower lid ulcerative blepharitis (6/15 in group E and 9/15 in group G). In addition, four of these mice (one from group E and three from group G had a mild punctate epithelial keratopathy. One mouse (group G) developed an encephalitis by day 10 and was killed. At 2 weeks there were varying degrees of lid scarring and hair loss (Fig 1) and two mice (group G) had developed an ipsilateral mydriasis.

Mice in groups F (scarified with strain McKrae) and H (scarified with strain SC16) developed disease as in group C. Two mice from group F and one from group H developed an encephalitis and were killed. No detectable difference in severity of disease was found between groups F and H.

Experiment 3

Mice developed varying degrees of blepharitis in a similar time course to the topically inoculated animals in experiment 2, although the disease was milder.

NEUTRALISING ANTIBODY RESPONSE

Sera from pre-infection and control mice (groups B and D) showed no detectable levels of antibody.

Experiment 1

Twenty-five of the 30 (83%) topically infected mice (group A) had detectable levels of antibody (Table 2) with a mean ED_{50} of 14·0 (range: 2 to 121) which was significantly lower than those scarified (group C, mean $ED_{50}=157·0$, range: 78 to 240 *t* test, $p<0·001$) (Fig 2a).

Experiment 2

Mice topically infected with strain SC16 (group G, mean $ED_{50}=237$, range: 26 to 575) had significantly higher levels of neutralising antibody than those topically infected with strain McKrae (group E, mean $ED_{50}=116$, range:2 to 401) (*t* test $p<0·05$). Conversely, mice infected by scarification with strain McKrae (group F, mean $ED_{50}=880$, range:234 to 2205) had a significantly higher mean ED_{50} than with strain SC16 (group H, mean $ED_{50}=330$, range:170 to 464 (*t* test, $p<0·05$), (Fig 2b.) There was no significant difference between the mean ED_{50} of

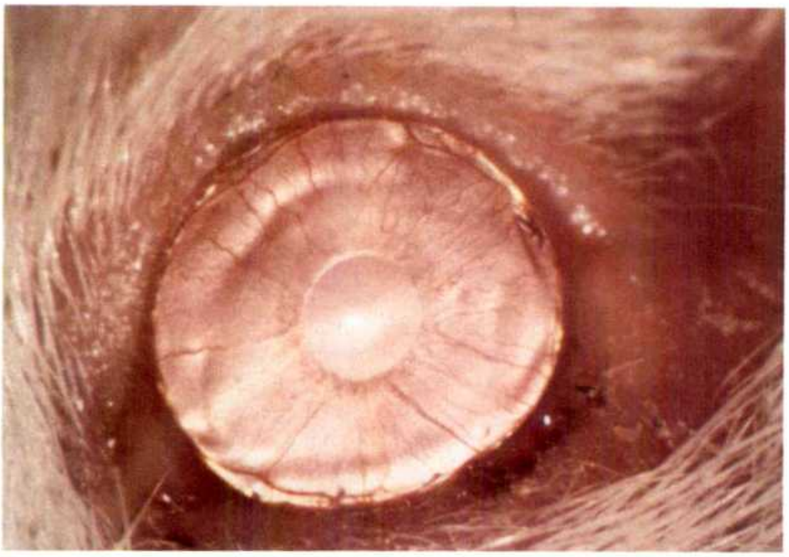

Figure 1 Clinical disease. *A 5½-week-old female NIH mouse, 2 weeks after topical inoculation with 10^{6} pfu of HSV-1 strain SC16. Note the presence of blepharitis, hair loss, an absence of corneal ulceration.*

Table 2 Polymerase chain reaction and ED_{50}

Mouse		*TK1*	*TK2*	*gC*	*ED_{50}*	*Mouse*		*TK1*	*TK2*	*gC*	*ED_{50}*
A1	C	+	+	+	8	B1	C	–	–	–	0
	I	+	+	+			I	–	–	–	
	TG	+	+	+			TG	+	+	–	
A2	C	+	+	+	5	B2	C	–	–	–	0
	I	+	+	+			I	–	–	–	
	TG	+	+	+			TG	–	–	–	
A3	C	+	+	+	3	B3	C	–	–	–	0
	I	+	+	–			I	–	–	–	
	TG	+	+	–			TG	–	–	–	
A4	C	+	+	+	28	C1	C	+	+	+	128
	I	+	+	+			I	+	+	+	
	TG	+	+	+			TG	+	+	+	
A5	C	+	+	+	5	C2	C	–	+	+	161
	I	+	+	+			I	–	+	+	
	TG	+	+	+			TG	+	+	+	
A6	C	+	+	+	121	C3	C	+	+	+	108
	I	–	–	–			I	+	+	+	
	TG	–	–	+			TG	+	+	+	
A7	C	–	–	–	0	C4	C	+	+	+	110
	I	–	–	–			I	+	+	+	
	TG	–	–	–			TG	+	+	+	
A8	C	–	–	–	0	C5	C	+	+	+	302
	I	–	–	–			I	+	+	+	
	TG	–	–	–			TG	+	+	+	
A9	C	+	+	+	33	D1	C	–	–	–	0
	I	+	+	–			I	–	–	–	
	TG	+	+	+			TG	–	–	–	
A10	C	–	–	–	0	D2	C	–	–	–	0
	I	–	–	–			I	–	–	–	
	TG	–	–	–			TG	–	–	–	
A11	C	–	–	–	6	D3	C	–	–	–	0
	I	–	–	–			I	–	–	–	
	TG	–	+	+			TG	–	–	–	
A12	C	+	+	+	2	U1	C	–	–	–	
	I	–	–	–			I	–	–	–	
	TG	–	–	–			TG	–	–	–	
A13	C	+	+	+	19	U2	C	–	–	–	
	I	+	+	+			I	–	–	–	
	TG	+	+	+			TG	–	–	–	
A14	C	–	+	+	47	A15	C	+	+	+	6
	I	+	+	+			I	+	+	+	
	TG	+	+	+			TG	–	–	–	
A16	C	+	+	+	2						
	I	–	–	–							
	TG	+	+	+							

TK1, TK2=primers for thymidine kinase; gC=primers for glycoprotein C; ED_{50}=neutralising antibody response; +=presence of PCR amplification product; –=absence of PCR amplification product; C=cornea; I=iris; TG=trigeminal ganglion; A1-A16=topical inoculation with 5×10^3 pfu HSV-1 strain McKrae; B1-B3=topical inoculation with mock inoculum; C1-C5=scarified with 5×10^3 pfu HSV-1 strain McKrae; D1-D3=scarified with mock inoculum; U1 and U2=uninfected mice.

mice topically infected with 10^6 pfu of strain SC16 and those scarified with 10^4 pfu of strain SC16 (p>0·1).

POLYMERASE CHAIN REACTION (EXPT 1)

Topical infection with virus

Thirteen of 16 mice had an antibody response to topical HSV-1 and evidence of HSV-1 DNA in their cornea and/or iris and/or TG (Tables 1 and 2). Of these 13 mice, viral DNA was detected using all three primers as follows: (a) in the cornea, iris, and TG of five mice (A1, A2, A4, A5, A13), (b) in two of the tissues in four mice (A9, A14, A15, and A16), and (c) in one of the tissues in three mice (A3, A6, and A12).

Both the TK1 and TK2, but not the gC amplification products were present in the iris and TG of A3, and only in the iris of A9. Conversely, the gC but not the TK amplification products were present in the TG of two animals (A6 and A15). These may represent genomic retention of false positives. Furthermore, there was evidence of HSV-1 DNA in the TG of A6 and in the cornea of A11 using the TK2 and gC primers, but not the TK1 primers. The absence of TK1 in the presence of TK2 and gC probably reflects the stronger signals obtained with TK2 and gC than with TK1, rather than incomplete HSV-1 DNA, as the TK2 product overlaps the TK1 product.

There was no significant difference in the number of positives that is, presence of all amplification products, in any of the tissues tested (Fischer exact test, p>0·1 for all).

Three mice with no detectable antibody response showed no evidence of HSV-1 DNA in the cornea, iris, or TG.

Scarification with virus. HSV-1 DNA was detected (using all three primers) in all tissues of each mouse in group C, with the exception of the iris and cornea of one animal (C2), where it could be detected using the TK2 and gC primers but not with the TK1 primers (Table 2).

Controls. There were no detectable amplification products using the three primers in the cornea, iris, or TG of eight control mice, apart from the presence of the TK amplification products in the TG of B1 (Table 1).

PRODUCT DIGESTION AND HYBRIDISATION

The amplification products (Fig 3a) were cleaved as predicted by the relevant restriction enzymes, confirming their identities (Fig 3b and c). Similarly, the TK1 and TK2 amplification products from positive samples hybridised to the internal oligonucleotide probe, whereas the negative control samples did not (Fig 3d).

ISOLATION OF VIRUS BY COCULTIVATION

Experiment 1

No virus was detected in cornea, iris, or TG (either cultured with or without 5′-azacytidine), from topically infected or control animals.

Virus was isolated from the TG of 5/6 scarified mice (group C) between days 10 to 14 (average day 12), but not from the iris or cornea.

Experiment 2

Virus was isolated from the TG of 5/14 mice topically infected with strain SC16 (average day 15) (group G), and from the TG of 3/15 mice infected with strain McKrae (average day 21) (group E), are of which also had virus cultured from its iris on day 23. Virus was isolated from the TG of 7/9 mice which had been scarified with strain McKrae (average day 14) and from the TG of 6/9 mice which had been scarified with strain SC16 (group H) (average day 15). In addition, virus was also isolated from the iris of two mice from the latter group on days 22 and 23 of culture.

No virus was isolated from the cornea in any of the above groups.

REACTIVATION

No virus was detected in eye washings taken from the tear film, either before irradiation or in the week following irradiation.

All mice (including the controls) developed UV induced diffuse punctate epithelial keratopathy with epithelial oedema and swollen eyelids 1 day after UV irradiation. By day 2 significant areas of epithelial loss had developed

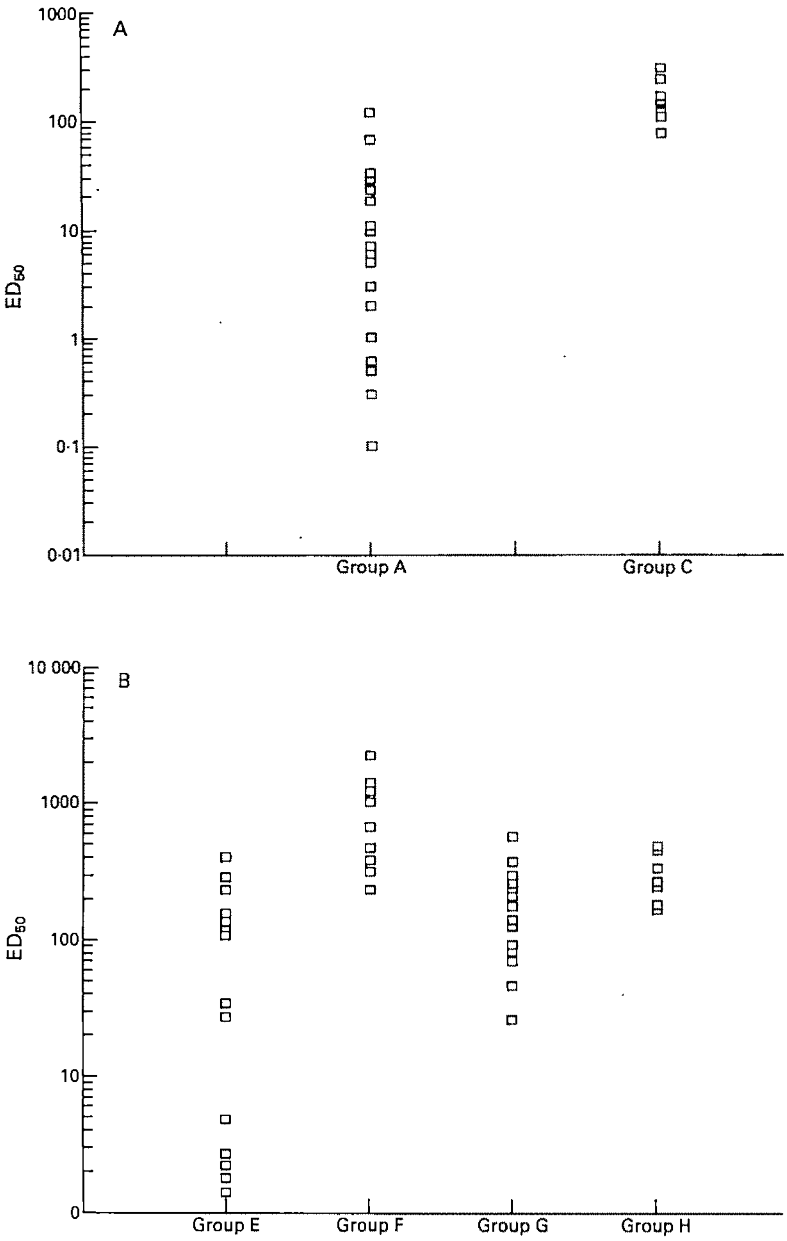

Figure 2 Neutralising antibody *response expressed as the ED_{50}. (A) (Expt 1): group A, topically inoculated with 5×10^4 pfu HSV-1 strain McKrae; group C, scarified with 5×10^3 pfu HSV-1 strain McKrae. (B) (Expt 2): groups E and G, topical inoculation with 10^6 pfu HSV-1 strain SC16 or McKrae respectively; groups F and H, scarified with 10^4 pfu HSV-1 strain SC16 or McKrae respectively.*

spreading across the cornea which was associated with mild iris hyperaemia. By day 3, the corneas of all control and 19/24 infected mice had re-epithelialised. Five of the 24 infected mice had persistent central oval epithelial defects and focal areas of lid excoriation. At 1 week, all the central epithelial defects had healed, two mice being left with scarred vascularised corneas.

Discussion

HSV-1 infection is usually acquired by direct or indirect contact spread from an infected individual. The non-traumatic acquisition of HSV-1 by direct droplet spread to the eye, however, has not been fully investigated. Asymptomatic ocular infection[17,18] has been postulated to occur based on the finding of anti-HSV-1 antibodies in the tear film of seronegative individuals[23] and although reports are contradictory, the isolation of HSV-1 in the tear film of persons with no prior history of herpes.[6,24]

Lower doses of HSV-1 strain McKrae did not produce disease after topical inoculation of older mice. Primary ocular disease did occur, however, following topical inoculation with either strain of HSV-1 (SC16 or McKrae), when higher doses were used in younger mice. This probably reflects the combination of increasing the dose of virus and the greater susceptibility of younger mice.[25] The incidence and severity of disease was slightly greater when SC16 was used for inoculation. For both strains of virus the disease was predominantly confined to the lids and resembled that which occurs in human primary ocular herpetic blepharitis.[17,18]

The pattern of disease (lid and corneal ulceration) in mice scarified with HSV-1 was similar to that reported previously.[12,14,26]

Topical application of HSV-1, induced the production of neutralising antibody in 83% of animals in expt 1 and in all animals in expt 2, irrespective of recognisable clinical disease. Mice which developed clinical disease, however, had significantly higher levels of antibody than those which did not. The level of antibody produced was highest in younger mice which had been scarified with HSV-1 strain McKrae. Interestingly, there was no significant difference in the level of antibody between mice which had been topically infected with 10^6 pfu of SC16 or scarified with 10^4 pfu of SC16, while there was a significant difference between mice topically infected with 10^6 pfu of McKrae and those scarified with 10^4 pfu of McKrae. These findings suggest that production of antibody following ocular infection is not only dependent on the dose of virus used, but also on the strain of virus and the method of inoculation.

A positive result for the likely presence of large stretches of HSV-1 DNA in a tissue was defined as the presence of both TK and gC amplification products. The presence of only one amplification product was taken to represent incomplete fragments of HSV-1 DNA (genomic retention or false positives), and a negative result defined as the absence of both the TK and gC amplification products. Using this definition HSV-1 DNA was present in the majority of mice that had been topically infected with HSV-1. There was a good correlation between the presence of HSV-1 DNA and the presence of detectable antibody in that only mice that had evidence of antibody were PCR positive. HSV-1 DNA has previously been detected in the TG and eyes of mice inoculated by scarification,[27] so that finding viral DNA in the TG, cornea, and iris or scarified mice was not unexpected. In the majority of tissues tested from mice topically infected or scarified with virus, all three amplification products were present, which indicates the likelihood that intact HSV genomes, rather than fragments of HSV-1 DNA, were present.

The isolation of HSV-1 between days 17 and 23, from the TG in 27% (8/29) of mice following topical inoculation (expt 2), suggests that a virus had been able to establish a latent infection. Although there were more isolations from the TG of mice inoculated with HSV-1 strain SC16 than with McKrae, the difference was not signi-

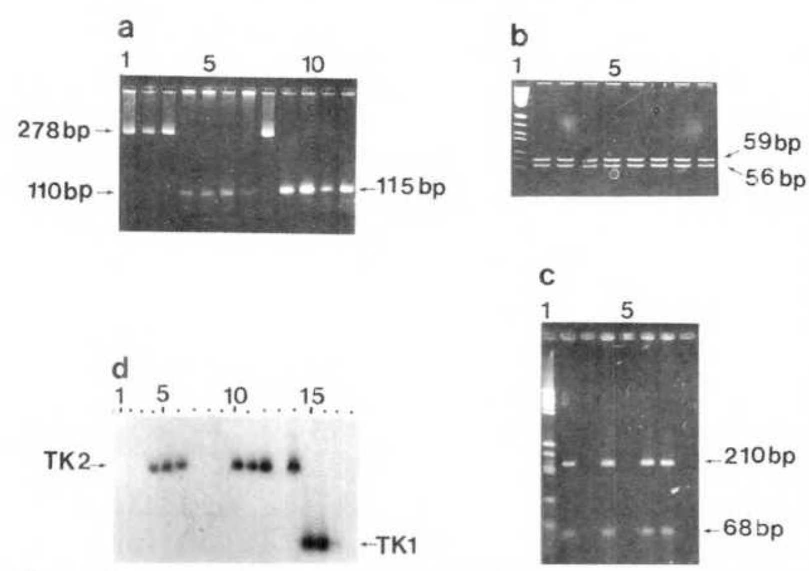

Figure 3 Polymerase chain reaction. *(a) 3% agarose gel. TK2 amplification product (278 bp): lanes 1–3 (cornea, iris, and TG, topical inoculation with 5×10^5 pfu HSV-1 McKrae), 8 (TG scarified with 5×10^3 pfu HSV-1 McKrae); TK1 amplification product (110 bp): lanes 4–6 cornea, iris and TG, scarified 5×10^3 pfu HSV-1 McKrae), 7 (cornea, topical inoculation 5×10^5 pfu HSV-1 McKrae), gC amplification product (115 bp): lanes 9 and 10 (cornea and TG scarified 5×10^3 pfu HSV-1 McKrae), 11 and 12 (cornea and TG, topical inoculation 5×10^5 pfu HSV-1 McKrae). (b) 12% polyacrylamide gel. Lanes 1–8, Ksp 1 digest of gC amplification product (59 and 65 bp), lane 9 (*Hae*111 digest of pBR322 plasmid DNA). (c) 3% agarose gel, Sma 1 digest of TK2 product (210 and 68 bp). Lane 1, marker (*Hae*111 digest of pBR322 plasmid DNA). (d) Autoradiograph 3 hours. 5′ end labelled probe from a 26 base oligonucleotide for TK1 (110 bp) and TK2 (278 bp) amplification products. Lanes 1–3 (TG, uninfected and mock infected), 4–6 (cornea, iris and TG, 5×10^5 pfu HSV-1 McKrae topical infection), 7–9 and 13 (gC amplification products), 10–12 (cornea, iris and TG, scarified with 5×10^3 pfu HSV-1 DNA McKrae), 14 (HSV-1 McKrae), 15 (TG scarified with 5×10^3 pfu HSV-1 McKrae), 16 (HSV-1 McKrae), 17 and 18 (TG, mock infected and uninfected).*

ficant. The isolation of virus from approximately 80% of the TG of scarified mice is similar to previous reports,[14,28] and probably reflects the greater virus load reaching the TG.

The inability to isolate HSV-1 from the central cornea of mice (where it was detected by PCR in 11/16 cases), is somewhat at odds with previous work.[14,29] The amount of virus in the 2 mm of cornea may have been below the sensitivity of cocultivation, (particularly following topical inoculation) but well within the sensitivity of the PCR. In previous reports (using scarification), although virus was isolated from the anterior segment[29] and cornea, the amount of tissue taken was not specified.[14] It is not clear therefore, whether virus may have been present in higher amounts in the peripheral cornea and limbus, conjunctiva, or iris, or in all three tissues. This is supported in the present study by the isolation of virus from the iris of three mice (one topical and two scarified) in the absence of corneal isolation.

Whereas virus was detected by cocultivation in the iris of 3/50 (6%) mice in expt 2, viral DNA was detected in the iris in 12/21 (56%) animals by PCR in expt 1. Although this may have been due to an absence of reactivatable virus, it probably reflects the greater sensitivity of the PCR, particularly in view of the higher doses of virus used for inoculation in expt 2.

UV irradiation induces shedding of virus in the tear film and recurrent eye disease in mice previously inoculated by scarification with HSV-1 strain McKrae.[22] In this study, after similar stimulation, we were unable to detect virus in the tear film of mice previously topically inoculated with 3×10^5 pfu of HSV-1 strain SC16. To increase the likelihood of induced reactivation in topically infected animals, further work is underway, varying the dose and strain of HSV-1 used for inoculation.

HSV-1 virus can establish a latent infection in the trigeminal and superior cervical ganglion in animals and humans.[30,31,32] In addition, recent work suggests that HSV-1 may also be able to establish a latent infection in the anterior segment of mice,[14,29] and the corneas of rabbits[33] and humans.[7,10,34,35]

Clearly, either in the presence or absence of overt ocular disease, ocular inoculation is the most direct way in which HSV-1 may reach these sites to establish a latent infection. In experimentally infected mice however, virus may also reach these sites, and establish latency in the ophthalmic division of the TG, by the 'back door route'[26] following a primary infection in non-ophthalmic parts of the trigeminal dermatome, for example the lower lid.

Following ocular inoculation neural spread of virus from tissues such as the cornea or eyelids seems the most likely route of the virus to the TG. Ocular infection, however, may involve a more complex pattern of spread.[36] Thus drainage of virus from the eye via the nasolacrimal duct can lead to infection via the olfactory route. Hence involvement of the TG might occur from the infected nasal mucosa via the nasociliary branches of the ophthalmic division.

The results of the present study show that, following non-traumatic topical application of HSV-1 to the mouse eye, virus accedes to the cornea, iris, and TG. By analogy in humans this might represent a symptomatic or asymptomatic primary infection, which leads to a latent infection of the TG and possibly of the iris and cornea. Ocular disease may thus potentially result from reactivation of virus at any of these sites of latency.

The clinical features and events following topical application of virus to the mouse cornea mimics primary ocular disease in humans and may prove useful for studying the pathogenesis of HSV-1 ocular disease.

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Endophthalmitis following extracapsular cataract surgery: a review of 32 cases

C J Heaven, P J Mann, D L Boase

Department of Ophthalmology, Queen Alexandra Hospital, Cosham, Portsmouth PO6 3LY
C J Heaven
P J Mann
D L Boase

Correspondence to:
C J Heaven.

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Abstract

Thirty two cases of endophthalmitis following extracapsular cataract surgery that had occurred within our department and had undergone intraocular diagnostic tap between May 1982 and May 1991 were reviewed. An infectious agent was identified in 20 cases (62·5%). The commonest organism was *Staphylococcus epidermidis* (11 cases) (55%). *Proteus* was the only gram negative organism identified (four cases) (20%). Both of these organisms were associated with a favourable visual outcome. In the culture positive subgroup 15 eyes (75%) achieved a final acuity of 6/60 or better with 10 eyes (50%) gaining 6/12 or better. Thirteen (65%) of the culture positive cases were managed without vitreal intervention. Of these 11 (85%) achieved 6/60 or better with eight (62%) gaining 6/12 or better. It appears that when an endophthalmitis follows uncomplicated extracapsular cataract surgery delivery of antibiotic by the 'conventional' routes (topical, subconjunctival and systemic) is consistent with a favourable visual result in many cases. A modified anterior chamber diagnostic tap technique is described.

Bacterial endophthalmitis is a potentially catastrophic complication of cataract surgery. Its management remains controversial. Dissatisfaction with the visual outcome from 'conventional' treatment using topical, subconjunctival, and systemic antibiotic has resulted in a trend towards the use of intraocular antibiotic injection and vitrectomy.[1-8] However this evolution in management has been largely based on the published experience of endophthalmitis following intracapsular cataract surgery and endophthalmitis occurring after other forms of ocular surgery or trauma.[2 3 5 9-14] There are relatively few reports of the visual outcome of endophthalmitis specifically following extracapsular cataract surgery.[15] We have therefore undertaken a review of cases of endophthalmitis occurring within our department following planned extracapsular cataract extraction.

Patients and methods

Operating theatre records were used to trace cases that had suffered an endophthalmitis following extracapsular cataract surgery and who had undergone anterior chamber and/or vitreous diagnostic tap. Thirty two such cases had occurred between May 1982 and May 1991. The medical records of each case were reviewed and the information extracted included: patient personal details, details of the cataract surgery, time of onset of endophthalmitis and presence of hypopyon, details of the intraocular diagnostic tap, additional surgical management, use and route of administration of antibiotics and corticosteroids, duration of intraocular inflammation, preoperative and final visual acuity, period of follow-up, and other co-existent ocular pathology.

In all cases the intraocular diagnostic tap specimen had been cultured on blood agar, chocolate agar, Sabouraud's media, and thioglycollate broth. A microbiological report was available for each case.

Results

In 20 cases (62·5%) a bacterial infection had been identified (culture positive). The spectrum and relative frequency of organisms is shown in Figure 1 and was similar to that reported in previous series.[2 3 5 7-13 16] A gram positive organism was identified in 16 cases (80%). All four cases (20%) of gram negative infection were due to *Proteus* species. The commonest organism was *Staphylococcus epidermis*, in 11 cases (55%). Twelve cases (37·5%) were culture negative. The culture positive and culture negative cases are subsequently considered as separate groups.

GROUP 1: CULTURE POSITIVE CASES

Individual case details are shown in Table 1. Of the 20 culture positive cases of endophthalmitis 12 were female and eight male. The age range was 41 to 91 years with a mean of 77 years. In nine the cataract surgery had been performed under general anaesthesia, while 11 had received a local anaesthetic of which five had been day cases. Nineteen of the 20 (95%) had undergone 'uncomplicated' extracapsular cataract extraction with posterior chamber intraocular lens implantation (ECCE+P/C IOL). In a single case (case 1) a planned ECCE+P/C IOL had been complicated by vitreous loss. This had necessitated an anterior vitrectomy and conversion to the use of an anterior chamber intraocular lens.

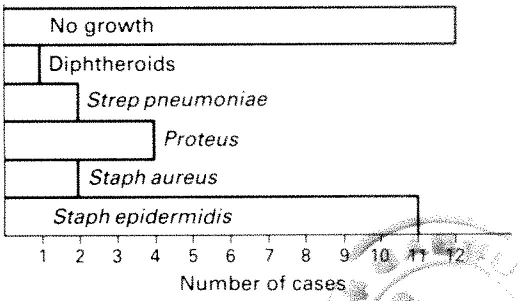


Figure 1 Incidence and spectrum of organisms identified from intraocular tap – all cases.

Table 1 Endophthalmitis after extracapsular cataract surgery. Culture positive cases

Case no	*Sex*	*Age*	*Anaesthesia*	*Day case*	*Presentation delay (days)*	*Hypopyon*	*Organism*	*Pre OP-V/A*	*Final V/A*	*(Antibiotic)* *Topical*	*Subconj*	*Systemic*	*Vitreous tap*	*Intravitreal antibiotic*	*Vitrectomy*	*Systemic steroid*	*Resolution (weeks)*	*Complication*	*Follow-up (months)*
1	F	82	GA	–	3	+	Staph epi	CF	CF	+	+	+	+	+	+	–	5	RD	84
2	F	89	GA	–	3	+	Diphths	CF	6/24	+	+	–	–	+	–	–	8	CMO	25
3	F	85	GA	–	2	+	Proteus	6/24	6/12	+	+	+	–	+	–	+	8	–	39
4	M	78	LA	–	4	+	Staph epi	CF	6/60	+	+	+	+	+	+	+	34	MP	14
5	M	88	LA	–	2	–	Strep pneu	6/60	NPL	+	+	+	–	–	–	–	Evisc	NA	20
6	M	84	GA	–	3	+	Staph epi	6/18	6/12	+	+	+	–	–	–	–	9	–	21
7	F	86	LA	–	2	+	Staph aur	HM	CF	+	+	+	–	–	–	–	10	–	24
8	M	41	GA	–	3	–	Staph epi	6/24	6/6	+	+	+	–	–	–	–	17	–	27
9	M	71	GA	–	2	–	Staph epi	HM	6/9	+	+	+	–	–	–	–	7	–	11
10	F	76	LA	+	3	+	Proteus	6/18	6/12	+	+	+	–	–	–	–	12	PS	19
11	F	72	LA	–	15	+	Staph epi	HM	CF	+	–	–	+	+	–	–	13	MP	21
12	F	85	LA	+	24	+	Staph epi	6/24	6/9	+	+	+	–	–	–	–	40	CMO	19
13	F	76	GA	–	3	+	Staph epi	6/36	6/18	+	+	+	+	+	+	+	20	–	14
14	F	78	GA	–	1	+	Staph aur	6/60	6/12	+	+	+	–	–	–	+	6	RD	14
15	M	70	LA	+	2	+	Staph epi	6/24	6/60	–	+	+	–	–	–	–	10	MH	3
16	F	91	LA	–	1	+	Strep pneu	6/36	HM	+	+	+	–	–	–	–	6	DC	5
17	F	89	GA	–	2	+	Proteus	CF	6/12	+	+	–	+	+	–	–	30	–	7
18	F	72	LA	+	1	+	Proteus	6/24	6/9	+	+	+	–	–	–	–	7	CMO	5
19	M	67	LA	–	5	–	Staph epi	6/36	6/9	+	–	+	–	–	–	–	8	–	5
20	M	58	LA	+	3	+	Staph epi	6/18	6/18	+	+	+	–	–	–	–	5	–	2

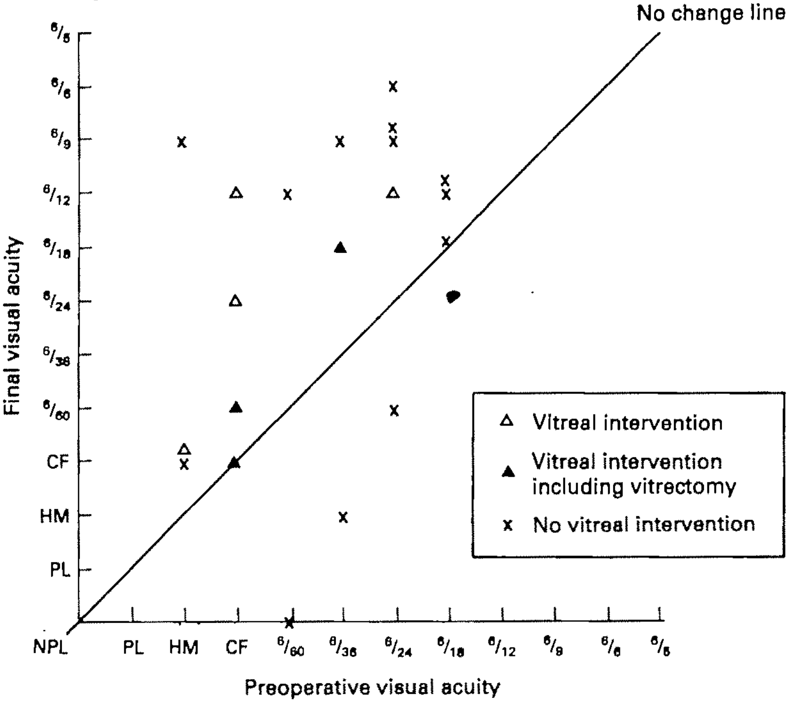
GA=general anaesthetic; LA=local anaesthetic; Staph epi=*Staphylococcus epidermidis;* Staph aur=*Staphylococcus aureus;* Strep pneu=*Streptococcus pneumoniae;* Diphths=Diphtheroids; RD=retinal detachment; CMO=cystoid macular oedema; MP=macular pucker; PS=pupillary seclusion; MH=macular hole; DC=decompensated cornea; NA=not applicable.

The time interval between cataract surgery and diagnosis of endophthalmitis (presentation delay) ranged from 1 to 24 days with a mean of 4 days. Following the onset of clinical endophthalmitis all cases underwent a diagnostic anterior chamber tap. In addition five had a pars plana vitreous tap. Three cases underwent pars plana vitrectomy. Seven cases received intravitreal antibiotic. In four of these gentamicin was used alone while three received a combination of antibiotics; (case 2: gentamicin+penicillin+methicillin, case 4: gentamicin+methicillin, case 13: gentamicin+cefuroxime+vancomycin). The dose of intravitreal gentamicin ranged from 0·1 mg to 0·3 mg. In no case was the intraocular lens removed. The duration of intraocular inflammation ranged from 5 to 40 weeks, mean 13 weeks. Follow-up was for between 2 and 84 months, mean 19 months.

Figure 2 is a scattergram which compares preoperative and final visual acuities for the culture positive group. In 17 cases (85%) the final vision was as good as or better than the preoperative acuity. Fifteen (75%) had a final acuity of 6/60 or better. Ten (50%) had a final acuity of 6/12 or better. A favourable outcome was associated with *Proteus* and *Staphylococcus epidermidis* infection and no vitreal intervention. Only one of the five vitreous taps and two of the seven instances of intravitreal antibiotic injection gained an acuity of 6/12 or better.

Five cases had a 'poor' visual outcome with final acuity less than 6/60. This tended to be associated with *Streptococcus pneumoniae* infection.[10] The two cases of *Staphylococcus epidermidis* endophthalmitis whose final acuity was worse than 6/60 had both undergone vitreous tap and intravitreal antibiotic and both had suffered ocular morbidity that could have been related to this intervention, namely retinal detachment and macular pucker.

Figure 2 Scattergram – culture positive cases, preoperative versus final visual acuity.



GROUP 2: CULTURE NEGATIVE CASES

Individual case details are shown in Table 2. Of the 12 culture negative cases seven were female and five male. The age range was 47 to 87 years, mean 68 years. In nine cases the cataract surgery was performed under general anaesthesia, while three received a local anaesthetic of which two had been day cases. In all 12 cases the extracapsular cataract surgery was 'uncomplicated' with the insertion of a posterior chamber intraocular lens. Ten cases suffered an endophthalmitis within 8 days of cataract surgery, mean 5 days. In all of these cases the intraocular inflammation eventually subsided taking from 6 to 32 weeks to do so, mean 12 weeks. However, in two cases (cases 27 and 30) the endophthalmitis was of late onset (90 and 44 days respectively) and in both the intraocular inflammation persists after prolonged follow-up. These two cases appear to have behaved in a manner typical of the recently described 'localised endophthalmitis' where microorganisms may be sequestered within the capsular bag resulting in negative diagnostic tap.[6 17]

Table 2 Endophthalmitis after extracapsular cataract surgery. Culture negative cases

Case no	Sex	Age	Anaesthesia	Day case	Presentation delay (days)	Hypopyon	Pre OP-V/A	Final V/A	(Antibiotic) Topical	(Antibiotic) Subconj	(Antibiotic) Systemic	Vitreous tap	Intravitreal antibiotic	Vitrectomy	Systemic steroid	Resolution (weeks)	Complication	Follow-up (months)
21	F	75	LA	+	4	+	6/18	6/9	+	+	–	–	–	–	+	6	–	10
22	F	70	GA	–	7	+	6/12	6/9	–	+	–	–	–	–	+	13	–	45
23	F	87	GA	–	6	+	6/60	6/9	+	+	+	–	–	–	–	13	–	20
24	F	53	GA	–	6	+	6/36	6/9	+	+	–	–	–	–	–	13	–	55
25	F	82	GA	–	6	+	6/12	6/9	+	+	+	–	+	–	+	32	–	34
26	F	57	GA	–	5	+	6/60	6/6	+	+	+	–	–	–	+	6	–	30
27	F	78	GA	–	90	+	6/60	6/18	+	+	+	+	–	+	+	*	–	30
28	M	75	LA	+	2	+	CF	PL	+	–	+	–	–	–	–	8	–	7
29	M	63	GA	–	8	+	6/60	6/5	+	+	+	–	–	–	–	4	–	31
30	M	47	LA	–	44	+	HM	6/9	+	+	+	–	–	–	–	*	–	60
31	M	73	GA	–	7	+	6/60	6/18	+	+	+	–	–	–	–	6	MP	7
32	M	53	GA	–	3	+	6/18	6/60	–	+	+	+	+	–	–	22	MP	35

*=Ongoing intraocular inflammation.

Figure 3 is a scattergram which compares preoperative and final visual acuities for the culture negative group. Eleven eyes (92%) had a final visual acuity of 6/60 or better. Eight (67%) attained a final acuity of 6/12 or better.

The culture negative group thus overall had a better visual outcome than the culture positive group. This is in agreement with previously reported experience.[9-12]

Discussion

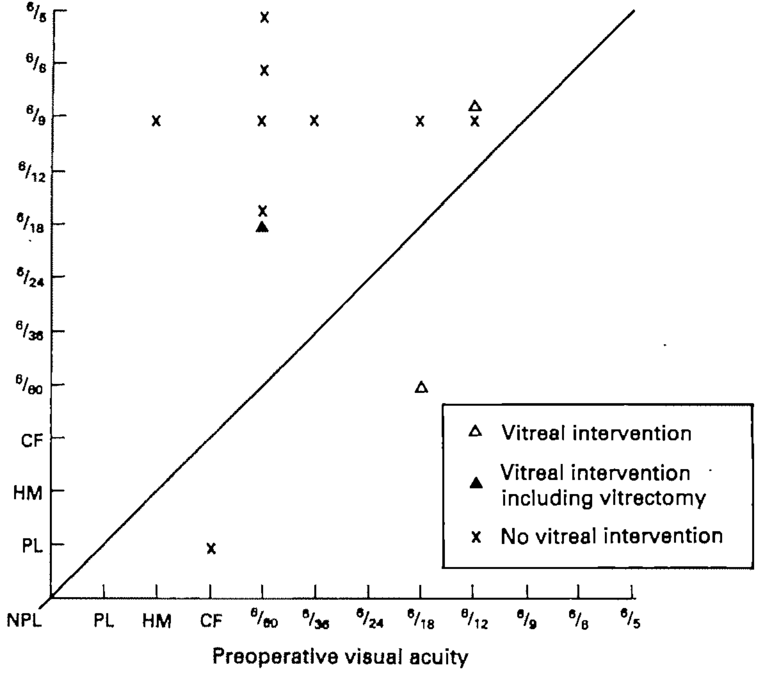
Historically the visual outcome from endophthalmitis using the 'conventional' treatment regimen of topical, subconjunctival, and systemic antibiotics was poor. Up to the late 1970s visual results of only hand movements or worse were reported to occur in 67% to 96% of cases.[1] In an attempt to salvage useful vision in a greater number of eyes direct injection of antibiotic into the vitreous and in some cases vitrectomy has been gaining favour in the management of endophthalmitis. A prospective study to determine the benefit of vitrectomy in endophthalmitis is currently in progress.[1]

Previously reported series of cases of endophthalmitis have considered its occurrence collectively after various forms of ocular surgery and trauma. In this context vitreal intervention has resulted in an improved visual outcome with those eyes gaining an acuity of 20/400 or better reported at between 42% and 73%.[2 3 5 7 9-12 18] Fewer eyes achieve a near normal acuity; 13% 20/40 or better,[2] 55% 6/15 or better.[18] However there have been relatively few reports of the visual outcome from endophthalmitis specifically when it occurs after extracapsular cataract extraction. Our series helps to fill this gap.

We show that bacterial endophthalmitis continues to be a potentially devastating complication of modern cataract surgery. Five (25%) culture positive cases failed to regain navigational vision in the eye affected (worse than 6/60). However overall the visual outcome for endophthalmitis following extracapsular cataract surgery compares very favourably with that reported for endophthalmitis occurring in other situations. In addition a good visual outcome may be achieved by 'conventional' treatment alone. Of the 20 culture positive cases 13 (65%) were managed without intravitreal antibiotic or vitrectomy. Only two (15%) of these resulted in a final acuity worse than 6/60; both were due to *Streptococcus pneumoniae*. Eight (62%) achieved an acuity of 6/12 or better. This relatively favourable outcome may be due to the high proportion of low virulence organisms. However the presence of an intact zonule and posterior capsule may alter the behaviour of an endophthalmitis. Bacteria introduced into the eye during extracapsular cataract surgery may be denied access to the vitreous by the intact capsulo-zonular diaphragm. Such a barrier effect has been demonstrated in primate studies.[19 20] In the human eye the posterior capsule has been shown to be a barrier to the diffusion of fluorescein from the posterior chamber into the vitreous.[21]

Vitreous intervention, whether by vitreous tap, intravitreal antibiotic injection, or vitrectomy, carries a risk of additional ocular morbidity. In our series one rhegmatogenous retinal detachment and three cases of macular pucker followed vitreous tapping and intravitreal antibiotic and each of these cases had a final visual acuity of 6/60 or worse. Gentamicin, either alone or in combination with other antibiotics, was used in every instance of intravitreal antibiotic injection. The retinal toxicity of intravitreal gentamicin has been demonstrated in animal eyes.[22 23] A 'safe' dose for injection into

Figure 3 Scattergram – culture negative cases, preoperative versus final visual acuity.



the human vitreous in the treatment of endophthalmitis has been determined empirically and that currently recommended is 0·1 mg.[24,25] However much of the published experience of the use of intravitreal gentamicin in cases of endophthalmitis following cataract surgery relates to intracapsular cataract extraction. The presence of an intact posterior capsule may alter the pharmacokinetics of antibiotic injected into the vitreous cavity. The clearance of drug from the vitreous to the anterior chamber may be prolonged. The half-life of a drug injected into the vitreous has been shown to be related to the degree of vitreal inflammation.[24-26] An intact posterior capsule may delay the establishment of vitreal inflammation in endophthalmitis after extracapsular cataract extraction. The appropriate dose for an intravitreal antibiotic therefore remains uncertain making its use problematic.

The antibiotics traditionally used in ocular infections, when administered topically, subconjunctivally or systemically, attain therapeutic concentrations within the anterior chamber but not within the vitreous.[25] In an endophthalmitis following uncomplicated extracapsular cataract surgery, where infection is primarily one of the anterior segment, 'conventional' management may be adequate if instigated aggressively and early and where the organism is of low virulence. The use of intravitreal antibiotic injection may be unnecessary and its potential complications thus avoided. Imipenem, a wide spectrum third generation cephalosporin administered intravenously, has been reported to achieve therapeutic levels in both aqueous and vitreous.[27] Its use may thus further reduce the need for vitreal intervention.

The commonest infecting organism, *Staphylococcus epidermidis* is associated with a relatively favourable visual outcome. Of our 11 cases nine (82%) achieved 6/60 or better with five (45%) gaining 6/12 or better. O'Day has reviewed *Staphylococcus epidermidis* endophthalmitis occurring after a variety of surgical procedures and found 78% achieved a final acuity of 20/50 or better by 'conventional' treatment.[13] When managed using intravitreal antibiotic and/or vitrectomy only 42% achieved 20/50 or better. However in all retrospective studies, including ours, there may be a bias towards the worst cases being managed by vitreal intervention.

In our series anterior chamber diagnostic tap was performed in all cases. This yielded a culture positive rate of 59% (19 of 32). This compares with a 56% to 77% culture positive rate for aqueous and/or vitreous tap previously reported in series of cases of endophthalmitis of mixed aetiology.[2 9 10 12 26] It has been noted that in such mixed cases of endophthalmitis a vitreous tap is more likely to be positive than an anterior chamber tap.[3 5 11-14] However in the series reported by Driebe *et al* the only two cases that were anterior chamber culture positive/vitreous tap culture negative were in eyes that had intact posterior capsules and posterior chamber lenses.[11] In our series five culture positive cases had both anterior chamber and vitreous taps. Four were positive for the anterior chamber tap of which two were also positive for the vitreous tap. One case was culture positive from the vitreous alone (case 11). We therefore consider that in cases of endophthalmitis following uncomplicated extracapsular cataract surgery where the primary focus of infection is in the anterior segment an anterior chamber tap alone is an adequate initial procedure.

In the light of the recently described 'localised endophthalmitis' wherein organisms are sequestered in the capsular bag[6] we have modified our anterior chamber tap technique. We no longer use a tuberculin type syringe as previously described in the literature.[12 16 28] It is our current practice to reopen the cataract section sufficient to insert an 18 gauge coaxial irrigation/aspiration cannula with a 0·3 mm sideport. With this we take two separate specimens for culture. The first tap is from the anterior chamber, teasing free any fibrinous membranes and including hypopyon where present. The second specimen is taken from within the capsular bag. This may require freeing up of the anterior capsular frill and some intraocular lens manipulation but with care to preserve the integrity of the posterior capsule and zonule. The tap specimens are applied to blood agar, chocolate agar, Sabouraud's media, and thioglycollate broth. The use of an additional enrichment broth, for example brain heart infusion, and specific solid media culture for anaerobes may further increase growth yield.[29 30] The procedure is concluded with a period of aspiration/irrigation to remove macroscopic debris. Irrigation is with balanced salt solution. The value of this approach is illustrated by case 20 where *Staphylococcus epidermidis* was cultured from the capsular bag tap while the anterior chamber tap was sterile. Liason with the microbiologist is essential in order than growth of low virulence organisms such as *Staphylococcus epidermidis* or *Propionibacterium* is not dismissed as a contaminant.

In no culture positive case did it prove necessary to remove the intraocular lens in order to resolve the infection or facilitate vitrectomy. However in the two culture negative cases with ongoing chronic uveitis a low grade infection within the capsular bag remains a possibility and ultimate removal of the capsular bag and intraocular lens may be necessary to achieve a quiet eye. Two cases (cases 4 and 12) were culture negative on the first anterior chamber tap but culture positive after repeat tap. It is our view therefore that all cases of endophthalmitis after extracapsular cataract surgery should be managed as infections even if culture negative.

It appears to us that endophthalmitis after uncomplicated extracapsular cataract extraction with posterior chamber intraocular lens implantation can be managed by anterior segment diagnostic tap alone and without recourse to vitreal intervention. Delivery of antibiotic by the 'conventional' topical, subconjunctival, and systemic routes is consistent with a favourable visual outcome in many cases and avoids further risks to the eye. It is our current policy only to consider vitreous tap, intravitreal antibiotics, or vitrectomy in cases of endophthalmitis following extracapsular cataract extraction where there had been capsular rupture or where there is overt evidence of vitreal infection.

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Possible environmental sources of *Acanthamoeba* spp in contact lens wearers

David Seal, Fiona Stapleton, John Dart

Abstract

The water supply and dust samples from the home environment (bathrooms and kitchens) of 50 wearers of contact lenses (CLs) were cultured for the presence of free-living amoebae. CL cases, solutions, and water taps were cultured for bacteria, which amoebae require for growth. *Acanthamoeba* spp were isolated from water drawn from six bathroom cold water taps (tank supplied), five in the presence of limescale, and from one kitchen cold water tap (mains supplied). There was an association between the presence of limescale in water and direct culture for free-living amoebae, suggesting that scale provides a favourable microenvironment for amoebae. *Acanthamoebae* were also found in dust from around one washbasin. Nineteen of 50 CL cases, 12/122 CL care rinsing solutions, and 59/100 cold water taps yielded Gram negative bacteria which could be ingested by amoebae. It is concluded from this study that CLs should not be washed in first-drawn tank-fed cold water, especially if limescale is present, and that soft CLs should be rinsed in manufactured single-use, sterile solutions. Rigid CL and CL cases should only be washed with boiled tap water (preferably hot), or single-use sterile solutions, and stored *dry* to prevent multiplication of amoebae and Gram negative bacteria.

Keratitis due to the free-living amoebae, *Acanthamoeba polyphaga* and *A castellanii*, occurs mainly in wearers of contact lenses (CLs),[1] but can also follow corneal trauma, often in rural surroundings.[2] The majority of patients have worn daily wear soft lenses,[3] but recent cases have occurred associated with disposable extended wear CLs.[4,5] The infection is painful, prolonged, difficult to treat, and can result in blindness.[6]

Acanthamoeba spp have been isolated from environmental sources including well water, hot tub and domestic tap water.[1,7-9] Although *Acanthamoeba* spp are ubiquitous in air, soil, and natural waters the main source of CL contamination in the UK has remained unclear for most patients. This contrasts with the USA, where the use of home-made saline for disinfecting CLs has been associated with *Acanthamoeba* keratitis.[1] A recent study of CL storage case contamination, involving asymptomatic CL wearers, has found *Acanthamoeba* to be present in seven of 102 cases, of which six also had high bacterial counts.[10]

To investigate possible sources for CL contamination, which is likely to occur prior to infection, 50 persons who wore CLs were visited at home. Samples were collected for isolation of amoebae from their bathrooms and kitchens including dust, the cold water supply, and the washbasin drain. These are the areas in the home where CLs are most likely to be handled. Cold water taps were also cultured for bacteria, as were CL storage cases and lens care solutions.

Materials and methods

PATIENTS

Fifty new patients who presented to the accident and emergency department at Moorfields Eye Hospital, wearing CLs for the correction of low refractive errors, were visited at home. Six had been admitted to the hospital with CL associated bacterial keratitis. Lens wearers without a problem associated with lens wear ($n=44$), who attended immediately following a wearer with bacterial keratitis, were identified as a control group.

HOME ENVIRONMENT SAMPLING

Cold water taps in each patient's bathroom and kitchen were initially sampled by inserting two swabs into each tap. Taps were not pre-heated and run to waste for 2 minutes, which is a standard public health technique to decontaminate the tap and to sample water in the supply pipe, as the requirement was to sample the tap flora likely to be deposited in a CL storage case. Dust in the bathroom was sampled with swabs behind the basin and in adjacent areas. Basin drains were sampled by inserting a swab through the plug hole and rubbing against the inside of the pipe. Swabs were processed for amoebae by placing them on non-nutrient agar seeded with live *Escherichia coli* washed in phosphate-buffered saline (PBS). Plates were incubated for 1 week at 37°C and observed for amoebae under low power microscopy. Identification was made on the basis of morphology and the presence of different types of cysts.

Water samples were collected from bathroom and kitchen cold water taps by drawing off 30 ml of first-run water through the tap, into a sterile bottle. Tap water was not run to waste initially. Thiosulphate was not added to the bottle, to neutralise chlorine in the water, again to simulate conditions in the CL storage cases. Water samples were spun at 2500 rpm for 10 minutes and 0·1 ml of the deposit was inoculated onto non-nutrient agar plates seeded with *Escherichia coli*, as above. These were incubated at 37°C for 1 week. Each sample was observed for the presence of limescale by microscopy of the spun deposit. These water samples were then reinvestigated by a more sensitive enrichment technique, which involved prior incubation of the water with live *Escherichia coli* for 48 hours. Samples were then

Institute of Ophthalmology and Moorfields Eye Hospital, City Road, London EC1V 2PD
D Seal
F Stapleton
J Dart

Correspondence to:
D Seal, Department of Bacteriology, Wolfson Centre, Level 5, 31 Taylor Street, Glasgow G4 0NA.

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plated out onto non-nutrient agar and incubated at 37°C. Different types of amoebae were identified by their morphological appearances.

CL storage cases were sampled with a swab which was rubbed around the inside of the case. These swabs, and others for bacterial culture, plus all lens care materials (3 ml of solutions), were cultured on 10% horse blood, MacConkey, and Sabouraud's agars. Bacteria were identified by standard methods. Patients were questionned as to whether and how CL storage cases were cleaned.

STATISTICAL METHODS

The χ^2 test with Yates correction factor, or Fisher exact test,[11] where appropriate, was used to analyse the results.

Results

Table 1 summarises the results for kitchen and bathroom water samples, including the presence of limescale, and culture for amoebae, *Acanthamoeba* spp, and bacteria. There was good association ($p<0\cdot001$) between isolation of amoebae by direct culture and the presence of limescale in cold water taps for both the kitchen and the bathroom. However limescale was more frequently found in bathroom taps ($p=0\cdot04$). Amoebae were found more commonly in the bathroom tap water (tank-fed tap) than in the kitchen water (mains-fed tap), by direct culture ($p<0\cdot05$). This association was lost when increased numbers of amoebae were recovered using the enrichment technique. *Acanthamoeba* spp were isolated more frequently (6/50) from bathroom water by the enrichment technique compared with kitchen water (1/50). This indicates a trend to growth for amoebae and *Acanthamoeba* spp in tank-fed water, a warmer environment, and in the presence of limescale. This difference was not statistically significant for *Acanthamoeba*, probably due to small numbers.

Amoebae and *Acanthamoeba* spp were not significantly associated with the presence of Gram negative bacteria in either water sample (Table 1) or tap swabs (data not shown), using direct or enrichment recovery techniques.

Amoebae were isolated from bathroom dust, beside the washbasin, on 6/50 occasions. On one occasion, *Acanthamoeba* sp was isolated as well.

Amoebae were isolated from the wash-basin drain on 40/50 occasions. Gram negative bacteria were present in 39 samples, of which amoebae were present on 30 occasions. Interestingly, amoebae were isolated from 10/11 samples when Gram negative bacteria were absent. *Acanthamoeba* spp were isolated on two occasions and Gram negative bacteria were present.

Sixteen CL cases belonging to 44 asymptomatic CL wearers were contaminated with Gram negative 'coliform' bacteria. Two out of five CL cases belonging to the patients who developed keratitis contained *Pseudomonas aeruginosa* while one CL case contained 'coliform' bacteria. Such 'coliform' bacteria were also isolated from 12 out of 122 CL care solutions overall. This contamination included 10 out of 108 CL care solutions from 44 asymptomatic CL wearers and two out of 14 solutions from six patients who had developed keratitis.

Results of questioning 35 asymptomatic CL wearers, who were resident at home, revealed that 13 washed their CL cases in tap water of which 11 used bathroom cold water and two used kitchen water. None ran their tap for 2 minutes prior to use but took off the first-drawn water. Fifteen did not clean their CL storage case while seven washed their cases in detergent.

Discussion

In England the cold water supply to the bathroom is derived from a storage tank in the roof that is rarely drained and cleaned. In contrast, the kitchen cold tap is supplied directly from the mains supply to the house, which later supplies the storage tank. *Acanthamoeba* spp, thought to be similar to pathogenic strains which infect the cornea, were isolated from 6/50 tank-fed bathroom cold water taps and from 1/50 mains-fed kitchen cold water taps. Isolation of amoebae and *Acanthamoeba* spp has been described from the water supply of Strasbourg,[12] which is derived

Table 1 · Association of amoebae and bacteria with limescale in kitchen and bathroom tap water

	Water samples					
	Bathroom (n=*50*)			*Kitchen* (n=*50*)		
	Limescale			*Limescale*		
Culture technique	*Present* (n=*32*)	*Absent* (n=*18*)	(*p*=*0·04*)*	*Present* (n=*21*)	*Absent* (n=*29*)	
Direct			p value†			p value
Amoebae§	24	0	<0·001 (χ^2=23) (p=<0·05)‡	12	1	<0·001 (χ^2=16)
Acanthamoebae	1	0	–	0	0	–
Bacteria¶ (tap swab)	19	13	NS	11	14	NS
Enrichment						
Amoebae§	29	15	NS	17	17	NS
Acanthamoeba	5	1	NS	0	1	–

*=comparison of the association of limescale and different sites
†=comparison of the association of amoebae with limescale
‡=comparison of the growth of amoebae in kitchens and bathrooms.
§=includes *Hartmanella, Naegleria, Vahlkamfia, Vanella, Vexillifera, Platyamoeba, Filamoeba* and *Nuclearia*
¶=Gram-negative rods
NS=not significant

from artesian wells. Free-living amoebae can also be expected to be found in the mains water supply in the UK. Furthermore, cysts of *Acanthamoeba* spp are relatively resistant to chlorine.[13]

Limescale was found more frequently in water samples drawn from bathroom cold water taps (32/50), compared with samples drawn from kitchen taps (21/50), $p=0{\cdot}04$. By direct culture, there was good association ($p<0{\cdot}001$) between the presence of limescale and amoebae, but this association was lost when the enrichment technique was used. Use of the enrichment technique, which allows small numbers of cells to multiply in the water sample prior to culture on agar, demonstrated that in 5/6 bathroom waters and in one mains water sample *Acanthamoeba* spp were present in low numbers, which could not be detected by direct culture from 0·1 ml of spun deposit derived from a 30 ml sample. There was no statistically significant correlation between the presence of bacteria and amoebae for either kitchen or bathroom samples, nor between bacteria and limescale. We propose that limescale traps the amoebae, which are known to survive in the absence of bacteria, by forming cysts. Small numbers demonstrated in the supplying water could then multiply in the limescale when bacteria are present. It would appear that free-living amoebae can be used as a marker for the possible presence of *Acanthamoeba* spp but are present from 6–24 times more frequently; *Acanthamoba* was not isolated in the absence of other amoebae. Our observation that only two cases of *Acanthamoeba* keratitis have been reported in Scotland (population 5 million).[14,15] which has 'soft' water without limescale, is interesting but needs confirmation and comparison with similar situations elsewhere. Both amoebae and *Acanthamoeba* spp were isolated from the washbasin drains and at a low frequency from the dust in adjacent sites.

Larkin *et al*[10] found that 43 CL storage cases, used by 102 asymptomatic wearers in an optometric practice, were contaminated by large numbers of bacteria and that seven contained *Acanthamoeba* spp. This present study has confirmed that CL storage cases from asymptomatic wearers are similarly contaminated with bacteria (16/44) in our population. This implies that CL disinfection in the community is ineffective. Furthermore, 12/122 commercial lens care solutions in the home were contaminated with Gram negative bacteria, despite being manufactured to remain sterile. In the USA a similar proportion of asymptomatic lens wearers has been reported as having bacterial contamination of their CL storage cases.[16]

Washing CL cases with freshly-drawn bathroom cold tap water, in areas using 'hard' water containing limescale, will deposit scale in them which is heavily contaminated with amoebae and bacteria. This scale may inactivate the chemical disinfectant process used. Organic debris has been identified as inactivating chlorine-based CL disinfectant systems.[17] These observations are consistent with the recent findings that six CL disinfecting solutions were not effective in eliminating *Acanthamoeba* cysts, from a starting suspension of 100 000, within a reasonable time for clinical use.[18] *Acanthamoeba* spp have also been shown to adhere to unworn hydrogel lenses,[19] which may further reduce the efficacy of chemical disinfectants.

Although a potential source of the organism has been from the lens storage case, two recent cases have been documented associated with the use of disposable extended wear soft lenses,[4,5] without the use of lens solution or a lens storage case. This demonstrates that other possible sources of the organism exist. These may include air,[20] dust such as that in the bathroom, shown in this study, swimming pools, and hot tubs.[21]

Amoebae ingest bacteria and thus good disinfection management involves the removal of both bacteria and amoebae. The findings of this study suggest that freshly-drawn bathroom cold tap water, particularly in 'hard' water areas, should *not* be used to wash out CL storage cases or to rinse the CLs themselves because of the high contamination rate. It is suggested that CL cases and hard CLs should be rinsed with freshly boiled and cooled water from the domestic kettle. CLs should be cleaned with a surfactant solution prior to disinfection, which should take place in a previously cleaned and dry case, ideally sterile and disposable. After disinfection the CL cases should be rinsed again with hot, boiled water and stored *dry* to reduce contamination and multiplication of amoebae and Gram negative bacteria. It must be accepted from this study and others[10,16] that current use of CL care systems by the general public is not as envisaged by the manufacturers nor as recommended by optometrists.

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Biometry of the crystalline lens in late onset diabetes: the importance of diabetic type

John M Sparrow, Anthony J Bron, Nicholas A Phelps Brown, H A W Neil

Abstract

Lenticular and anterior chamber biometry were studied in non-cataractous eyes by means of Scheimpflug photography and digital image analysis. The study population consisted of 91 late onset diabetic subjects and 115 non-diabetic controls. Anteroposterior axial lens thickness, cortical thickness, nuclear thickness, anterior clear zone thickness, anterior chamber depth, and anterior and posterior lenticular curvatures were assessed. Age played an important role in determining lens biometry in all subjects, and small but significant differences were found between late onset diabetics and non-diabetics. In the late onset diabetic subgroup, apart from age, diabetic retinopathy was the only significant parameter found which determined lens biometry. These biometric findings in late onset diabetes are in marked contrast to the large overall effect of diabetes and the powerful effect of diabetic duration which we previously reported in early onset diabetes. Further analysis of the data from our previous study has been provided, which clearly demonstrates differences between the impact of early and late onset diabetes on the biometry of the anterior ocular segment.

Mitotic activity in the pre-equatorial lenticular epithelium results in steady growth of the human crystalline lens throughout life.[1-5] Overall lens growth is determined by a dynamic balance between external accretion of secondary lens fibres and the central compaction of older nuclear or perinuclear fibres.[3 6] Anteroposterior lens growth is proportionally greater than equatorial growth, anterior and posterior lens curvatures therefore become steeper (shorter radii of curvature) with increasing age.[5 7]

Lenticular biometry is disturbed in diabetes,[5 8-10] with early onset diabetics showing markedly abnormal lens growth with a powerful dependency upon diabetic duration.[5] The anterior clear zone (first zone of disjunction) of the lens behaves independently of the other lenticular zones. This zone, which is neither age dependent nor dependent upon diabetic duration, has been found to be markedly increased in early onset diabetics.[5] The increased anterior clear zone thickness and the powerful dependency of other biometric parameters on diabetic duration imply that the lenses of early onset diabetics may not simply be over-hydrated, but that they may be in a state of accelerated growth, with either more (hyperplastic mechanism) or individually larger (hypertrophic mechanism) secondary lens fibres being formed. Intracellular 'swelling' of individual lens fibres would be theoretically possible although such swelling would need to be confined to the newly formed (metabolically active) fibres to be compatible with the powerful duration effect observed in early onset diabetes.[5] In order for extracellular swelling of the lens to explain the observed effect of duration it would be necessary to postulate that such swelling increased over time with diabetic duration. This is conceivable, as ongoing cumulative damage to membrane structures could take place over periods of years.

Previous studies have not examined specifically the effect of late onset diabetes on lenticular biometry.[5 9 10] The present study examines biometry in clear lenses of late onset diabetics and controls, and draws comparisons between the impact of diabetes on the lens in late and early onset diabetes.

Material and methods

SUBJECTS

A total of 91 late onset diabetics (57 males) and 115 non-diabetic controls (64 males) were included in the study. These patients formed part of a population-based comparative lens study.[11] The diabetics were survivors of the Oxford Community Diabetes Study,[12] and community-based controls were selected to group match the diabetics by age and sex. In the present study only the late onset diabetics were included. Late onset diabetics were regarded simply as all those diabetics who did not fit the inclusion criteria used in our earlier study for early onset diabetes. In effect this meant that late onset diabetics were defined as any diabetic whose age at onset was more than 30 years regardless of the type of diabetic treatment, or diabetics whose age at onset was 30 years or less, but who did not require continuous insulin treatment. Controls were all community based, and were accepted if they had no history of diabetes or impaired glucose tolerance, and a non-fasting whole venous blood glucose of less than 7·8 mmol/l.[13 14]

Individual eyes of diabetics and controls were included in the present study if the lenses were non-cataractous, and had normal anterior ocular segments and vision sufficiently good to hold fixation during Scheimpflug photography. Cataractous lenses were excluded because such lenses may have abnormal biometry,[2 15 16] although certain minor opacities as defined by the Oxford Clinical Cataract Classification and Grading System[17 18] were accepted: nuclear brunescence and white nuclear scatter up to and including Grade 2, spoke opacities and waterclefts of Grade 1, and isolated vacuoles, retrodots and focal dots. No anterior or posterior sub-

Nuffield Laboratory of Ophthalmology, University of Oxford
J M Sparrow
A J Bron
N A Phelps Brown

Department of Public Health and Primary Care, University of Oxford
H A W Neil

Correspondence to:
Dr John M Sparrow, Department of Ophthalmology, University of Leicester, The Leicester Royal Infirmary, Leicester LE1 5WW.

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capsular opacities were permitted, as subcapsular opacities were specifically known to be associated with reduced lens size.[15] There were thus a total of 378 eligible eyes in 206 subjects; 188 were right eyes and 190 were left eyes.

The study was approved by the Central Oxford Research Ethics Committee (ref no 1211), and informed consent was obtained from participants.

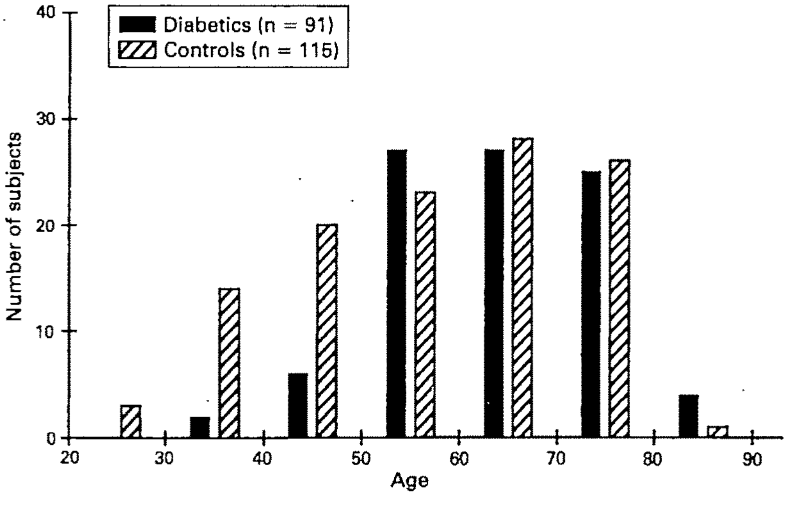
PROCEDURE

The procedure followed has been described.[5] Briefly, subjects were contacted and recruited by the primary investigator (JS). Following a short history and Snellen acuity testing pupils were dilated (tropicamide 1% and phenylephrine 10%) with due regard to the usual precautions. Lenses were assessed for cataract at the slit-lamp biomicroscope, and Scheimpflug photographs were taken using a Brown Scheimpflug camera.[19-21] Biometric measurements on digitised images were performed by a masked observer (JS) using the Oxford Modular Cataract Image Analysis System which was developed by the authors.[22] As in our previous study of lens biometry in early onset diabetes[5] the biometric parameters measured were: anteroposterior axial lens thickness, cortical thickness, nuclear thickness, anterior clear zone thickness, anterior chamber depth, and anterior and posterior lenticular curvatures. Cortical thickness was derived arithmetically as the difference between lens and nuclear thickness.

STATISTICAL METHODS

Biometric measurements were in general obtained from both eyes, although certain patients contributed measurements on only one eye. A preliminary analysis was performed by plotting the biometric data against age and performing simple linear regression for the diabetic and non-diabetic groups separately. In the main analysis the within-subject intereye correlation was accounted for by employing the intraclass correlation model of Rosner for calculating significance levels and confidence intervals.[23,24] The dependent variables were continuous and were approximately normally distributed. The main analysis took the form of a multiple linear regression analysis with groups (factors), the biometric measures being used as dependent variables in a series of model fitting exercises. Adjustment for minor imbalances in the case/control age distribution was achieved by treating age as a covariate in the relevant analyses. To facilitate comparison with our earlier study of lens biometry in early onset diabetes the analyses were performed in broadly the same manner as in that study.[5] (The orders of the fitting of terms to the models are indicated in the tables.) Using the data from the previous study as well as the data from the present study additional analyses were performed comparing the overall effects of early and late onset diabetes with non-diabetic controls, and comparing early and late onset diabetics directly. These analyses provide the average group differences (with 95% confidence intervals) between both types of diabetics and controls, and between early and late onset diabetics, after taking account of the (confounding) effects of age and sex. The analysis was performed using the Generalised Linear Interactive Modelling (GLIM) System of the Numerical Algorithms Group (NAG, Wilkinson House, Jordan Hill Road, Oxford OX2 8DR).

Figure 1 Frequency distribution of the ages of late onset diabetics (cases) and non-diabetics (controls).



Results

The age distribution of the late onset diabetics and controls is presented in Figure 1. The age/duration distributions for the diabetics alone are presented in Table 1. Figures 2 to 8 illustrate the preliminary regression analyses of biometry against age. These graphs demonstrate the important effect of age on anterior segment biometry. For certain of the biometric features studied there appear to be small differences between the late onset diabetics and controls.

These impressions were investigated in the formal analysis summarised in Table 2. The 'late onset diabetics and controls' analysis (206 subjects, 378 eyes) confirmed that age was an important determinant of biometry. Modest differences existed between diabetics and non-diabetics for axial lens thickness, cortical thickness, and front radius of curvature. These changes were such that lenses of diabetics were 'thicker' with steeper front curvatures. The significant age by diabetic status interaction term (age.sta) for front radius of curvature indicated that the younger diabetics were relatively more affected, this being well demonstrated in Figure 7 (regression lines non-parallel). A substantial difference in anterior clear zone thickness between diabetics and non-diabetics was found, with diabetics having increased clear zone thick-

Table 1 Age by diabetic duration of the late onset diabetes study population

	Diabetic duration (years)				
Age (years)	*<10*	*10 to <20*	*20 to <30*	*30+*	*Total*
30 to <40	2	0	0	0	2
40 to <50	4	2	0	0	6
50 to <60	10	13	4	0	27
60 to <70	10	14	1	2	27
70 to <80	6	11	7	1	25
80+	2	0	2	0	4
Total	34	40	14	3	91

ness. Female sex was associated with decreased cortical thickness.

In the late onset diabetic subgroup (91 subjects, 161 eyes) age was an important determinant of lens biometry for all parameters except nuclear thickness and anterior clear zone thickness. Proliferative diabetic retinopathy was associated with increased lens and cortical thickness. Diabetic duration, sex, or type of diabetic treatment (insulin, oral hypoglycaemics, or diet) did not have a demonstrable effect on any of the biometric parameters studied.

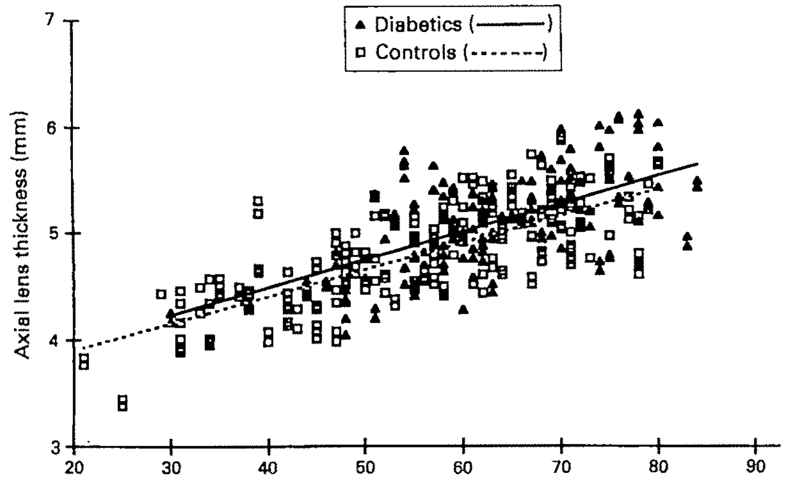


Figure 2 Plot of anteroposterior axial lens thickness derived from image analysis of Scheimpflug photographs against age. Separate linear regression lines for diabetics and controls.

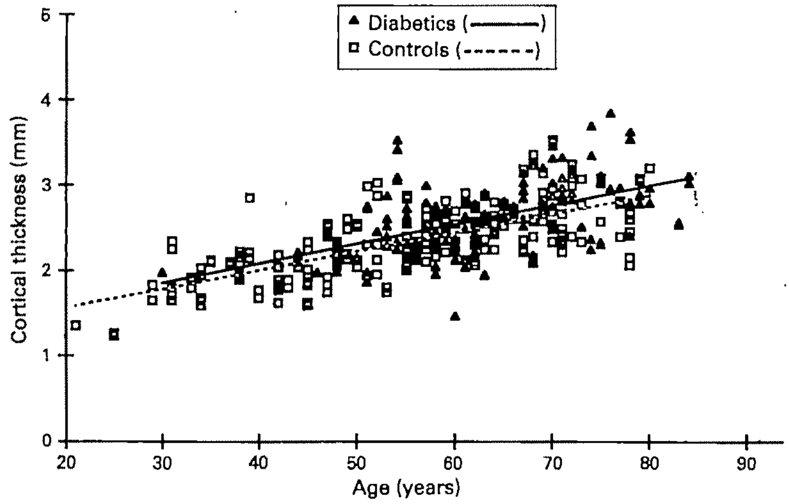


Figure 3 Plot of anteroposterior cortical thickness of the lens derived from image analysis of Scheimpflug photographs against age. Separate linear regression lines for diabetics and controls.

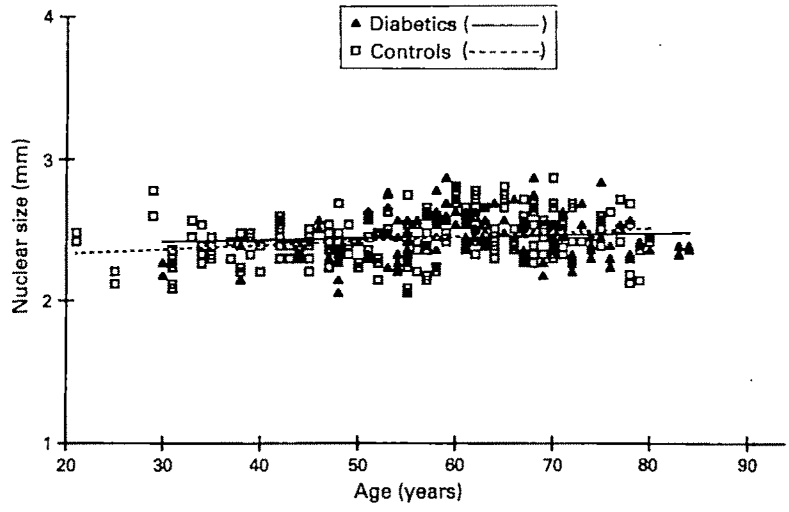


Figure 4 Plot of anteroposterior nuclear size of the lens derived from image analysis of Scheimpflug photographs against age. Separate linear regression lines for diabetics and controls.

Among the controls (115 subjects, 217 eyes) age was an important determinant of biometry, with female sex associated with decreased axial length and cortical thickness and with increased nuclear thickness.

The differences between the late onset diabetics and controls were observed to be less impressive than those previously found for 153 early onset diabetics and 153 controls[5] (the 115 controls in the present study being a subset of the previous 153). For this reason further analyses making a direct comparison between early and late onset diabetics were performed. The first of these analyses is summarised in Table 3, and demonstrated important differences between the two groups of diabetics (after accounting for confounding effects). The differences between the early and late onset groups were such that the lenses of the early onset patients were significantly larger in their axial, cortical, and nuclear thicknesses, had steeper front and back radii of curvatures, and were associated with shallower anterior chambers. The interaction terms between age and type of diabetes (age.type) indicated certain differences in the slopes of the regression lines between the two groups. The regression lines for lens thickness (cortical thickness) and front radius of curvature were each less steep in the late onset diabetics than in the early onset diabetics, indicating that the average change per year in biometry was greater in the early onset group.

The magnitudes of the observed effects (with 95% confidence intervals) are presented in Table 4. These analyses demonstrated the average effects of late onset and early onset diabetes on lens biometry compared with controls, and also compared early and late onset diabetics directly. After adjustment for age and sex, the effect sizes in the early and late onset diabetics compared with controls indicated that the impact of lens biometry of early onset diabetes was between two and three times that found in late onset diabetes. The final group of analyses in Table 4 provide direct estimates (adjusted for age and sex) for the overall differences between early and late onset diabetics. These estimates show that diabetics demonstrate a particular pattern of disturbance of anterior segment biometry, and that the magnitude of this disturbance is considerably greater among early onset than among late onset diabetics.

Because the early onset diabetics from our previous study[5] were on average younger than the late onset diabetics in the present study, two further analyses were performed to determine whether the observed differences might be due to differences in the age structures of the two diabetic populations. To examine this point attention was directed to the impact of diabetic duration, which, as has been noted above, was a powerful effect among the early onset diabetics,[5] but which appeared to play no role in the late onset diabetics. Firstly, the early onset group was examined to determine whether the powerful duration effect persisted across the entire age range. For this the early onset diabetics (age range 10 to 74 years) were grouped into age quartiles. The duration effect for the biometric parameters was then determined separately for each age quartile in a model which pre-fixed the age effect to that of the non-diabetic control group, and maintained a constant y-intercept for the age quartiles. A summary of this analysis appears in Table 5, which gives the duration estimates (and 95% confidence intervals) for each quartile. This analysis demonstrated that in general the duration effect among the early onset diabetics persisted across all the age quartiles. In the second analysis all the diabetics (early and late onset) were grouped in deciles according to their age at onset of diabetes. This approach dispensed with the prior classification of the diabetes into early and late onset groups. After adjusting for age and sex each 'onset decile' group was examined separately for an effect of diabetic duration (Table 6). These analyses demonstrated that for most parameters the effect of diabetic duration on biometry diminished dramatically when age at onset of diabetes exceeded 30 years.

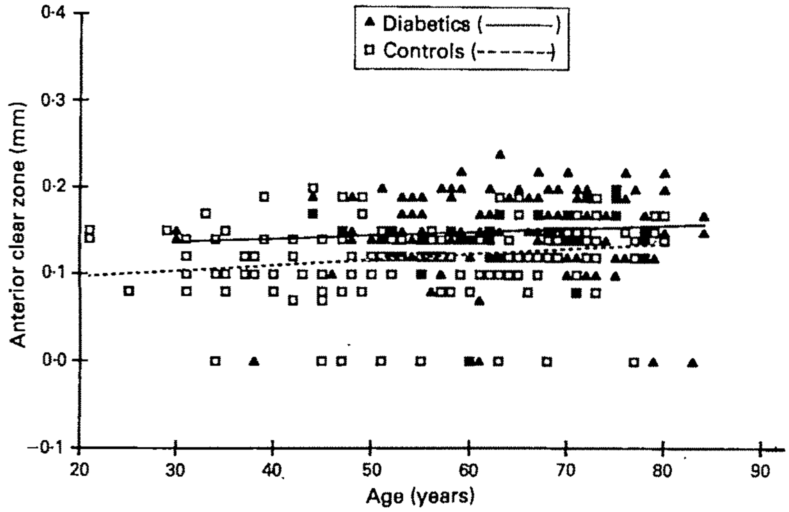


Figure 5 Plot of anterior clear zone thickness of the lens derived from image analysis of Scheimpflug photographs against age. Separate linear regression lines for diabetics and controls.

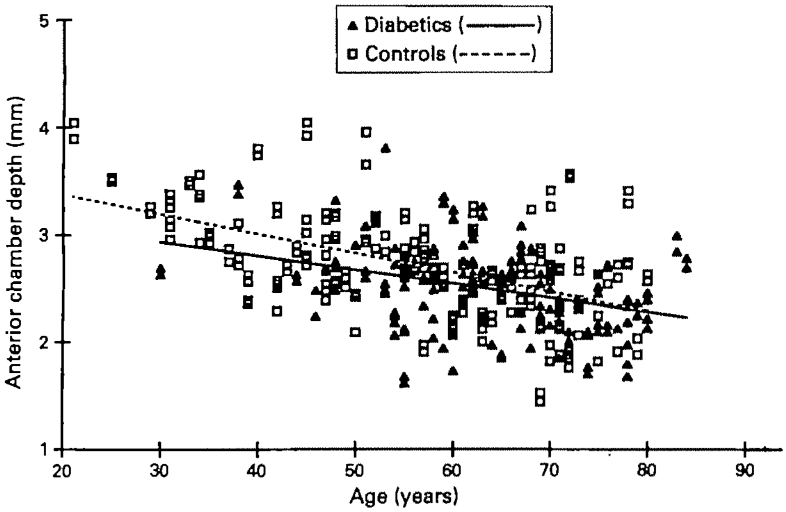


Figure 6 Plot of axial anterior chamber depth of the eye derived from image analysis of Scheimpflug photographs against age. Separate linear regression lines for diabetics and controls.

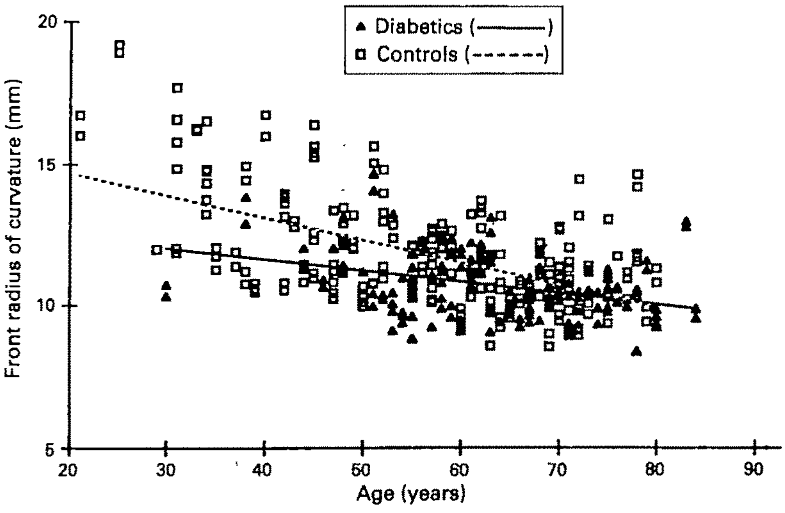


Figure 7 Plot of front radius of curvature of the lens derived from image analysis of Scheimpflug photographs against age. Separate linear regression lines for diabetics and controls.

Discussion

The results of this study have demonstrated important differences in the impact of late onset and early onset diabetes on the biometry of the anterior ocular segment. The impact of late onset diabetes on biometry was modest, and a direct comparison between early onset and late onset diabetics confirmed these biometric differences between the two groups of diabetics. Comparisons between each diabetic type and non-diabetic controls revealed that the impact on biometry of early onset diabetes was two to three times greater than the impact of late onset diabetes. Furthermore, within the diabetic subgroups, no effect of diabetic duration was found among the late onset diabetics, which was in marked contrast to the powerful effect of diabetic duration previously found in early onset diabetes.[5] Further analysis of the data from our earlier study has demonstrated that the duration effect in early onset diabetes persists across all the age quartiles (age range 10 to 74 years). The demonstration that the duration effect persists across the age quartiles is important, because it illustrates that the absence of a duration effect in the late onset diabetics cannot be attributed to the fact that the late onset diabetics were on average older than the early onset diabetics. When the classification of diabetics into early and late onset subjects was dispensed with, age at onset of more than 30 years was noted to be associated with a marked reduction in the effect of diabetic duration after adjustment for the effects of sex and ageing. These findings support the idea that the response of the lens to early onset diabetes is distinct, and is not simply a function of the age of the affected individual. It may be argued that the failure to demonstrate an effect of diabetic duration in the late onset diabetics could be due to the fact that disease duration is frequently not precisely known in such patients. The disease duration of such patients however would in general be underestimated and this would tend to amplify any actual association with duration. Our failure to identify any association with disease duration in

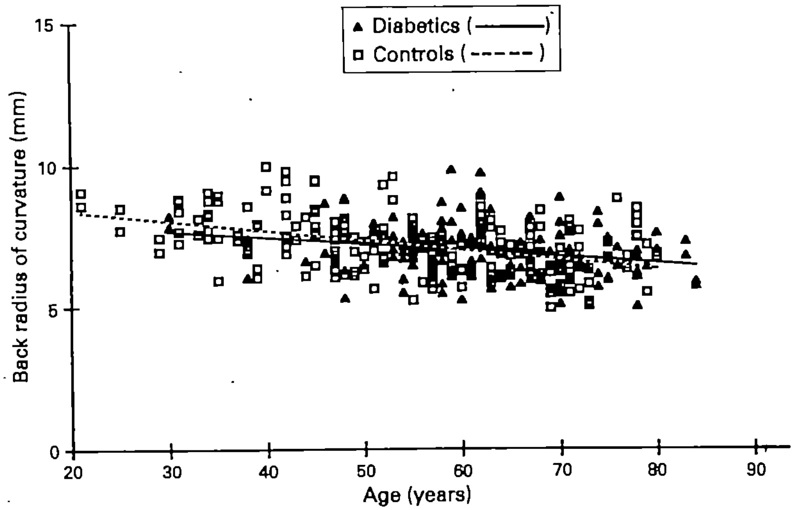


Figure 8 Plot of back radius of curvature of the lens derived from image analysis of Scheimpflug photographs against age. Separate linear regression lines for diabetics and controls.

Table 2 Summary of p values from analysis of lens dimensions in late onset diabetes and controls (clear lenses)

	Lens	*Cortex*	*Nucleus*	*ACZ*	*AC*	*FR*	*BK*
Late onset diabetics and controls (subjects=206, eyes=378, right=188, left=190)							
Age	$\ll 10^{-4}$	$\ll 10^{-4}$	0·0020	0·00031	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$
Sta	0·029	0·034	NS	0·000049	NS	0·0017	NS
Age.sta	NS	NS	NS	NS	NS	0·023	NS
Sex	NS	0·020	NS	NS	NS	NS	NS
R for model	0·73	0·69	0·24	0·35	0·53	0·60	0·43
Late onset diabetes (subjects=91, eyes=161, right=81, left=80)							
Age	$<10^{-4}$	$<10^{-4}$	NS	NS	0·0010	0·00029	0·0033
Dur	NS	NS	NS	NS	NS	NS	NS
Sex	NS	NS	NS	NS	NS	NS	NS
Treat	NS	NS	NS	NS	NS	NS	NS
BGR	NS	NS	NS	NS	NS	NS	NS
PLR	0·011	0·034	NS	NS	NS	NS	NS
R for model	0·67	0·63	0·26	0·22	0·37	0·45	0·41
Controls (subjects=115, eyes=217, right=107, left=110)							
Age	$\ll 10^{-4}$	$\ll 10^{-4}$	0·0024	0·014	$<10^{-4}$	$<10^{-4}$	$<10^{-4}$
Sex	0·024	0·00097	0·033	NS	NS	NS	NS
R for model	0·76	0·74	0·32	0·22	0·55	0·57	0·49

ACZ=anterior clear zone thickness of lens; AC=anterior chamber depth; FR=front radius of curvature of lens; BK=back radius of curvature of lens; Sta=diabetic or non-diabetic status; Dur=diabetic duration; Treat=diabetic treatment (diet, oral hypoglycaemic or insulin); BGR=background retinopathy; PLR=proliferative retinopathy; p values calculated by the intraclass correlation model of Rosner.[23 24]

Table 3 Summary of p values from analysis of lens dimensions in early and late onset diabetes (clear lenses)

	Lens	*Cortex*	*Nucleus*	*ACZ*	*AC*	*FR*	*BK*
Early and late onset diabetics (subjects=244, eyes=456, right=229; left=227)							
Age	$\ll 10^{-4}$	$\ll 10^{-4}$	0·00033	NS	$\ll 10^{-4}$	$\ll 10^{-6}$	$<10^{-4}$
Sex	NS	NS	NS	NS	NS	NS	NS
Type	$<10^{-4}$	0·000001	0·00021	NS	0·0010	0·000033	0·00029
Age.type	0·039	NS	0·052	NS	NS	0·0021	NS
R for model	0·82	0·80	0·34	–	0·63	0·63	0·37

ACZ=anterior clear zone thickness of lens; AC=anterior chamber depth; FR=front radius of curvature of lens; BK=back radius of curvature of lens; Type=type of diabetes (early or late onset); p values calculated by the intraclass correlation model of Rosner.[23 24]

Table 4 Comparison of the overall effects (±95% CI) of diabetes on lens biometry (mm) between controls and diabetics (late and early onset separately), and between early and late onset diabetics, after accounting for the (confounding) effects of age and sex (non-sequential analysis)

	Lens	*Cortex*	*Nucleus*	*ACZ*	*AC*	*FR*	*BK*
Late onset vs controls (n=206)	0·12* (0·093)	0·11* (0·091)	0·003 (0·042)	0·026* (0·012)	−0·11 (0·11)	−0·71* (0·39)	−0·022 (0·23)
Early onset vs controls (n=306)	0·37† (0·089)	0·28† (0·084)	0·099* (0·040)	0·049† (0·011)	−0·31† (0·10)	−1·93† (0·45)	−0·65† (0·21)
Early vs late onset (n=244)	0·43† (0·14)	0·31* (0·13)	0·11* (0·059)	0·16 (0·19)	−0·25* (0·15)	−1·32* (0·62)	−0·62* (0·33)

ACZ=anterior clear zone thickness of lens; AC=anterior chamber depth; FR=front radius of curvature of lens; BK=back radius of curvature of lens; 95% CI=95% confidence interval; *=$p<0·05$; †=$p<10^{-6}$; confidence intervals and p values calculated by the intraclass correlation model of Rosner[23 24]; +ve values indicate that the parameter is greater in diabetics *cf* controls in the first two analyses, and that the parameter is greater in early onset diabetics *cf* late onset diabetics in the 3rd analysis.

late onset patients is therefore unlikely to arise from inaccurate knowledge of true diabetic duration among late onset diabetics. The statistical power of this study to detect a 'medium' sized duration effect among the late onset diabetics in a multiple regression analysis (13% of the variance by Cohen's convention) at the 5% probability level for 91 subjects is 87%, and for 161 eyes is 99%.[25] The true power of the study as analysed by the intraclass correlation model[23 24] will therefore lie between these upper and lower limits, depending on the actual within-subject intereye correlation for each parameter under study. Even if the intereye correlation was high it would be reasonable to suppose that the power of this study to detect a medium size effect at the 5% level is at least 90%, which confirms that any duration effect among the late onset diabetics would have had to be small (and therefore of doubtful importance) to have been missed.

Our definitions of early onset diabetes as diabetes diagnosed at or before 30 years of age and requiring continuous insulin treatment, and late onset diabetes as all other diabetics have the effect of separating off a group of young subjects who almost certainly had Type 1 diabetes. This definition of Type 1 diabetes, although based purely on clinical features and age at onset, has been used extensively in epidemiological studies.[26 27] A prospective study of 268 newly diagnosed diabetic patients classified on the basis of clinical criteria only found that all patients diagnosed before the age of 40 years who were clinically assessed as insulin-dependent, had fasting C-peptide levels 18 months after diagnosis which were diagnostic of insulin dependence.[28] The classification that we used will therefore have ensured that early onset patients were correctly characterised as having minimal endogenous insulin production, although the late onset group is likely to have contained a small number of misclassified patients with true Type 1 (insulin-dependent) diabetes. This might confound comparisons between the early and late onset groups, and it is conceivable (although unlikely) that the 'weak' effects of diabetes seen in our late onset group are (partly) due to the misclassification and inclusion of a small number of truly Type 1 diabetics within a group which should ideally contain only Type 2 patients. Thirty (33%) of the late onset diabetics were using insulin at the time of the study, 15 (16%) having had their diabetes diagnosed at age <50 years, and 10 (11%) at age <40 years. The number of potential misclassifications was therefore not very great.

In the present study proliferative diabetic retinopathy was found to be associated with greater lens and cortical thickness, an effect which is in general consistent with our previous finding in early onset diabetics of increased clear zone and nuclear thickness, and steeper front and back curvatures in patients with retinopathy (confounding variables accounted for in each study). These associations raise the possibility that disturbances in lens biometry could be due to the forward diffusion of an (angiogenic) growth factor from the posterior ocular segment, or they may simply imply that patients with a

Table 5 Slope estimates (±95% CI) for the effect of diabetic duration (mm/year) on biometry in early onset diabetics across the age quartiles after accounting for the (normal) effect of age

Age quartile	*Duration estimate (95% CI)* *Lens*	*Cortex*	*Nucleus*	*AC*	*FR*	*BK*
1st	0·020* (0·011)	0·019* (0·010)	0·0018 (0·0054)	−0·023* (0·013)	−0·187† (0·055)	−0·045* (0·028)
2nd	0·019† (0·007)	0·016* (0·007)	0·0033 (0·0034)	−0·022† (0·008)	−0·132† (0·033)	−0·030* (0·017)
3rd	0·016† (0·005)	0·013† (0·005)	0·0029* (0·0025)	−0·016† (0·006)	−0·084† (0·025)	−0·016* (0·013)
4th	0·018† (0·006)	0·014† (0·006)	0·0038* (0·0026)	−0·011* (0·007)	−0·056* (0·029)	−0·013 (0·017)

95% CI=95% confidence interval; AC=anterior chamber depth; FR=front radius of curvature of lens; BK=back radius of curvature of lens; Age quartiles: 1st=10 to <26; 2nd=26 to <42; 3rd=42 to <58; 4th=58 to 74 years; *=$p<0·05$; †=$p<10^{-6}$; confidence intervals and p values calculated by the intraclass correlation model of Rosner.[23,24]

Table 6 Effect of diabetic duration on lens biometry in groups with different age of onset of diabetes after accounting for the (confounding) effects of age and sex

Age at onset of diabetes (number of subjects)	*Lens*	*Cortex*	*Nucleus*	*AC*	*FR*	*BK*
0 to <10 (43)	$<10^{-6}$*	0·000003	0·097	0·000016	$<10^{-6}$	0·000008
10 to <20 (60)	$<10^{-6}$	0·000068	0·00021	0·0092	0·000003	0·0036
20 to <30 (43)	0·000001	0·000004	0·057	0·0025	0·000013	0·039
30 to <40 (19)	NS	NS	0·066	NS	0·068	NS
40 to <50 (30)	NS	0·038	NS	NS	NS	0·051
50 to <60 (29)	NS	NS	NS	NS	NS	NS
60+ (20)	NS	NS	NS	NS	NS	NS

AC=anterior chamber depth; FR=front radius of curvature of lens; BK=back radius of curvature of lens; *p values calculated by the intraclass correlation model of Rosner.[23,24]

'worse' diabetic state tend to be concurrently affected by lens changes and retinopathy. As discussed in our previous publication,[5] a disturbance of the actual growth of the lens (by either a hyperplastic or hypertrophic mechanism or both) would be consistent with the finding in early onset diabetes of a powerful diabetic duration effect. With the modest changes found in late onset diabetes, and the absence of any demonstrable effect of diabetic duration, it is necessary to question whether the mechanisms of the disturbances to the lens are different in the two types of diabetes. The argument in favour of a disturbance of actual lens growth in early onset diabetes is partly based upon the finding of a powerful diabetic duration effect in this group, and is perhaps supported indirectly by the observation that biometry is preferentially disturbed in subjects with diabetic retinopathy. In the absence of a duration effect the slightly increased lens thickness in late onset diabetes could be explained simply in terms of an ion/water imbalance with generalised (extracellular) lens swelling. The idea that lenticular swelling might increase over time (duration) with cumulative membrane damage in early onset diabetes but not in late onset diabetes seems intrinsically unsatisfactory, and argues against 'progressive swelling' of the lens as the mechanism of disturbed biometry in early onset diabetes. Differences between early and late onset diabetics are well recognised, with proliferative diabetic retinopathy.[29] being commoner, and end stage renal failure[14,30] being about 15 times more common in Type 1 than Type 2 diabetics. Cardiovascular complications on the other hand are commoner among Type 2 diabetics.[14,31]

We conclude that in the present study substantial differences in anterior ocular segment biometry have been demonstrated between early and late onset diabetics. These biometric findings may reflect fundamental pathophysiological differences between Type 1 and Type 2 diabetes.

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MINI REVIEW

Nystagmus in infancy

Spontaneous nystagmus in infants may be present at birth but more frequently appears in the first 6 months.[1] We present a classification in which nystagmus with an onset before 6 months is called early onset nystagmus (EON) and is divided into three categories: sensory defect nystagmus (SDN) in which there is a proven sensory impairment, congenital idiopathic nystagmus (CIN) (sometimes called 'motor nystagmus'[2]) in which no visual or neurological impairment can be found, and neurological nystagmus (NN) which is associated with neurological disease. Though the distinction between SDN and CIN is made by many authors,[3-6] they are sometimes collectively called congenital nystagmus. The presentation of all types of nystagmus can be similar, but in infants it is important to differentiate between SDN and CIN because of the different underlying defects, investigation, management, and genetic counselling (Fig 1).

Congenital idiopathic nystagmus

CIN is diagnosed after exclusion of any underlying defect. It normally affects acuity moderately,[7,8] and estimates of its incidence vary from 1 per 350 to 1 per 20 000,[9-11] reflecting the different definitions of 'congenital nystagmus' and ways of data collection.

TYPICAL CIN

Typically, CIN is conjugate and purely horizontal even in depression and elevation.[12,13] The nystagmus intensity may decrease on convergence or with voluntary eyelid closure, and it may increase or decrease with fixation effort. There is frequently a region of gaze direction where the nystagmus is minimal or absent; this null region may be eccentric, in which case a head turn may be adopted, or it may be in the primary position in which case the nystagmus may go unnoticed for years. The waveform may be pure jerk, pure pendular, a mixture, or both jerk and pendular in different gaze directions. The nystagmus is highly variable between patients, and it often subsides with age.[14,15] CIN may have a hereditary component – as an X-linked trait with variable expressivity and incomplete penetrance, as an autosomal dominant characteristic, or as a sporadic occurrence.[16,17]

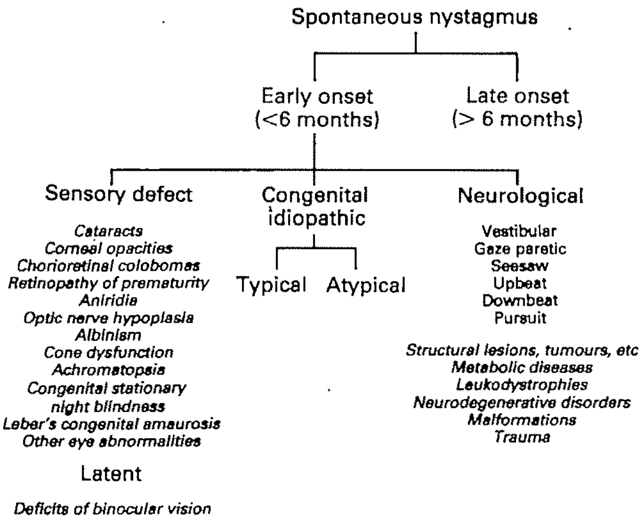

Figure 1 The classification of nystagmus with onset in infancy. The term 'early onset nystagmus' (EON) describes all *nystagmus with an onset before 6 months and is divided into three: sensory defect nystagmus (SDN) describes nystagmus associated with demonstrable anterior visual pathway disease including latent nystagmus; congenital idiopathic nystagmus (CIN) describes nystagmus not associated with any demonstrable visual or neurological impairment (except for poor acuity directly attributable to the nystagmus). Neurological nystagmus (NN) describes nystagmus not in the other two categories and usually associated with neurological disease.*

In infants with CIN normal optokinetic nystagmus is not usually recognisable. It has been reported that the quick phases of optokinetic nystagmus are sometimes in the reverse direction[18]; however, we have found this to be uncommon in infants and young children and clinically impossible to analyse. The vestibulo-ocular reflex is also often completely disrupted.[19] Infants can track moving objects with a varying nystagmus waveform superimposed provided the amplitude of the nystagmus is not too large. A completely normal child presenting with a typical CIN, an acuity of 6/18 or better, and no other neurological or eye defects can be diagnosed as having CIN without other investigations.

ATYPICAL CIN

The absence of the typical features by no means rules out the possibility of CIN. The diagnosis of atypical CIN is made by exclusion of an underlying defect and electrophysiological oculomotor, and neurological investigations should be performed. Neuroradiology, of which magnetic resonance imaging is usually the preferred technique in infants,[20,21] is indicated in patients with a normal electroretinogram and an abnormal visually evoked response or as a part of a neurological work-up. When the nystagmus is monocular or very asymmetrical, and there is a head tilt or turn and a head shake, the diagnosis of spasmus nutans may be made: this is a rather unhelpful diagnosis because it is retrospective. There must eventually be improvement and it is without predictive factors.[22] Since monocular nystagmus can be associated with serious intracranial pathology, investigations are essential as in other cases of atypical CIN.

Sensory defect nystagmus

SDN is the most common form of EON and is secondary to a variety of obvious or subtle disorders.[23-25] Cataracts, corneal opacities, optic disc atrophy, and developmental disorders of the optic disc and retina are among the commoner causes that are associated with visible abnormalities. Others have grossly normal eyes, such as Leber's amaurosis, achromatopsia, and congenital stationary night blindness. Albinism is a common cause that may be included in this group. These congenital disorders require differentiation by clinical findings, family history, laboratory tests, radiology, and detailed electrophysiological findings under photopic and scotopic conditions.[21,26] Visual evoked potentials reflect macular function and provide a means of objectively assessing visual acuity in infants,[27,28] and have specific features in albinism.[29-32]

The diagnosis of SDN is difficult when the sensory defect is subtle – for example, in a group of 64 children with EON, 29% had achromatopsia but 40% of this group were first diagnosed as having CIN.[33] Surveys of EON show the presence of sensory defects to be high (82%,[23] 88%,[24] and 91%[25]). SDN is sufficiently common for an electrophysiological test to be the initial test.[34] Even if there is no direct family history other members of the family should be examined to help establish the diagnosis and prognosis and for genetic advice to be given. The presence of a family history of a similar form of nystagmus does not preclude that nystagmus being SDN.

Neurological nystagmus

When the nystagmus is asymmetrical or unilateral, neurological disease should be suspected and a neurological consultation may be indicated.[35] Visual pathway disease giving rise to nystagmus includes chiasmal and optic nerve glioma, craniopharyngioma, and optic nerve compression by other tumours or bone anomalies. Many patients with NN also have associated neurological symptoms or present as very sick children with vomiting and headaches due to elevated intracranial hypertension.

Systemic disorders associated with nystagmus include Down's syndrome, hypothyroidism, maple syrup urine disease, and Pelizaeus-Merzbacher disease.[36-38] Monocular nystagmus is sometimes associated with epileptic seizures.[39] Seesaw nystagmus is readily identifiable and is usually secondary to a suprasellar defect (although an idiopathic seesaw nystagmus has been reported in an older child[40]). Early onset vertical nystagmus is often associated with toxic causes[41] or intracranial space-occupying lesions and demyelinating disorders,[38] though it can sometimes be idiopathic.[42]

Latent nystagmus

LN is a binocular jerk nystagmus which is absent with both eyes open. It may be detected in infancy and it usually becomes manifest on occlusion or blurring of the eye. The fast phase beats in the direction of the viewing eye. It is often associated with amblyopia and it is also found in conjunction with SDN in which case a complex nystagmus wave form is seen to change on occlusion.[43] LN may be manifest in patients with alternating strabismus in which case the nystagmus fast phases always beat in the direction of the fixating eye. LN may be always manifest[44] but this can be detected only by detailed studies.

Waveforms analysis in early onset nystagmus (Fig 2)

Nystagmus has been categorised clinically into jerk and pendular waveforms thought to correspond to CIN and SDN.[2,6] It is difficult to distinguish between waveforms clinically. Eye movement recordings have revealed 12 waveforms associated with SDN and CIN[45] consisting of variants of pendular, jerk, and mixed jerk-pendular types, with no correlation between waveform and aetiology.[45,46] In hereditary CIN affected individuals within the same family may have different waveforms,[47] and even affected monozygotic twins can have different waveforms.[48,49] Thus, it is not possible to differentiate between CIN and SDN on the basis of the nystagmus alone. Eye movement recordings have shown some clinically useful findings: in patients with rod monochromacy the horizontal nystagmus in centre gaze often has pendular and jerk waveforms with much lower amplitude than that in patients with other forms of SDN[50]; in infants, typical CIN is purely horizontal but SDN is often complex and multidirectional (often with a torsional component). The finding of jerk nystagmus with an accelerating slow phase does not distinguish between SDN and CIN but it does exclude NN. Acquired accelerating slow phase jerk nystagmus, caused by a cerebellar lesion, has been described in adulthood but is extremely rare.[51] Jerk nystagmus with decelerating slow phases is usually associated with latent nystagmus[44] or gaze paretic nystagmus.

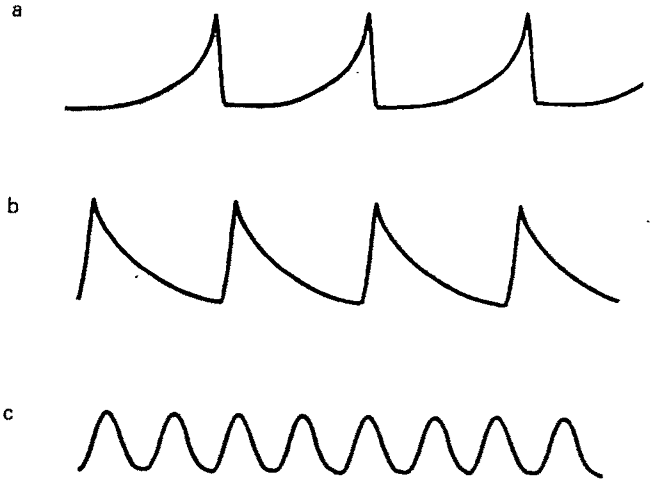

Figure 2 Illustration of the three basic slow phase profiles seen in early onset nystagmus. (a) Accelerating slow phase is almost always associated with SDN or CIN. (b) Decelerating slow phases are usually associated with latent nystagmus or gaze paretic nystagmus, and sometimes SDN or CIN. (c) Pendular nystagmus with no quick phases can be associated with CIN, SDN, or NN.

Management

All patients require refraction and spectacles if appropriate. At school age, educational needs should be taken into account, including sitting at an appropriate place in the classroom, informing the teachers that an abnormal head posture and head shaking may be useful adaptive behaviours which help the child to see better. Since many of these children are also strabismic, occlusion and other strabismus management are important.

In older children and adults improvement in visual acuity resulting from a decrease in nystagmus has been reported with baclofen,[52,53] 5-hydroxytryptophan therapy,[54] biofeedback,[55,56] contact lenses,[57] prisms,[58] and surgical procedures.[55-67] Most of these treatments have not stood the test of time.

Treatment directed at the nystagmus itself to improve visual acuity was first described by Bietti and Bagolini.[59] They performed a large recession of the four horizontal muscles. This technique has enjoyed a recent revival[60,61] but is not yet fully evaluated.[62]

Moving a null point nearer to primary position obviates the need for the head turn: this can be accomplished with prisms for small head turns.[63] Surgery for an unacceptable head position has been performed in very young children.[64,65] Factors other than the null zone influence the final head posture and were studied by Abadi.[66] Kestenbaum performed equal recessions and resections with shifting of the eyes in the direction of the rapid phase of the nystagmus, away from the null point.[67] Anderson recessed the muscles responsible for the slow component of the nystagmus,[68] and Goto resected the appropriate muscles without a corresponding recession.[69] Pratt-Johnson recommended symmetrical surgery on all four horizontal recti when there was no other associated strabismus present.[70] Parks suggested recession of one medial rectus 5 mm and a resection of the lateral rectus 8 mm with the opposite eye receiving a recession of the lateral rectus 7 mm and resection of the medial rectus muscle 6 mm.[71] Because of the high rate of recurrence Calhoun and Harley recommended no surgery in a 15 degree head turn and increased the amount of the 'classic Parks' surgery by 40%, resulting in surgery of 7, 8·4, 9·8 and 11·2 mm for a 30 degree head turn and a classic plus 60% for a 45 degree head turn.[72] Taylor recommended an 8 to 9 mm recession of the lateral rectus muscle on the side of the slow phase of the nystagmus and a 6 mm recession of the medial rectus muscle of the opposite eye in conjunction with 6 mm resection of the antagonists.[73] The recommended dosages of surgery reported by Scott and Kraft[64] are: (1) classic maximum for 20 degree turn; (2) classic plus 10% to 40% for 25–45 degrees turn; (3) classic plus 40% for 45 degrees or more. Turns over 50 degrees require augmentation of 50% to 60% to obtain an excellent result. Mitchell *et al*[65] reviewed the records of 48 patients with nystagmus and head turn. They were subdivided in six groups receiving different types of surgery. The results of this series is comparable to the series reported by Scott and Kraft,[64] and by Nelson.[74]

It does not require excessive cynicism to wonder why, when each report claims success, that successive publications recommend ever more surgery, even to the point of creating a gaze palsy.[64 72] Null point surgery should be reserved for those with genuine and significant symptoms.

Pathophysiology

The mechanisms underlying SDN and CIN are not known. Defects involving the saccadic system, the optokinetic system, the fixations system, and the neural integrator for conjugate horizontal eye movements have been proposed.[51 75 76] Optican and Zee[77] have succeeded in modelling most of the waveforms of CIN/SDN with a defect in the integrator. They suggest that the variable onset results from a congenital miswiring of proprioceptive signals which may only manifest as gaze instability when the infant learns to fixate. However, the association of SDN with such a wide range of apparently unrelated anterior visual defects points to a maladaptation to deprived macular vision during a critical period of visual development, rather than to an inborn structural anomaly. This is supported by reports of SDN occurring secondary to corneal opacities known to be acquired neonatally.[76 78] On the other hand, a maldevelopment during a critical period does not account for CIN that appears at birth or occasionally much later in life.[79]

Conclusion

It is important to classify EON correctly. SDN and NN, with subtle abnormalities, are often mistaken for CIN. Good clinical examination, family history, electrophysiology, eye movement recordings, and if necessary, neuroradiological and neurological examination should be considered in every infant presenting with nystagmus. The more rigorous investigations can be spared in cases of SDN with obvious defects or in typical CIN. By using a clear clinically relevant terminology, incorrect and confusing labels can be minimised. The term 'congenital nystagmus' should be avoided in describing EON since it has variable use.

I CASTEELS
C M HARRIS
F SHAWKAT
D TAYLOR

Eye Movement Unit,
Department of Ophthalmology,
Hospital for Sick Children,
Great Ormond Street,
London WC1N 3JH

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CASE REPORTS

Primary malignant ectomesenchymoma of the orbit

Thomas H Matsko, Rodney A Schmidt, Ann H Milam, James C Orcutt

Department of Ophthalmology
T H Matsko
A H Milam
J C Orcutt

Department of Pathology, University of Washington, Seattle, USA
R A Schmidt

Correspondence to:
James C Orcutt, MD,
Department of Ophthalmology RJ-10,
University of Washington,
Seattle, WA 98195-0001,
USA.

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Abstract

Malignant ectomesenchymoma (MEM) is a rare soft tissue tumour believed to arise from a pluripotential migratory neural crest cell and composed of both a mesenchymal element (most often rhabdomyosarcoma) and a neuroectodermal element (often neuroblastoma). Reported sites of origin are the abdomen, perineum or scrotum, the extremities, the middle ear, nasopharynx, face, and neck. We report the first case of an orbital MEM, with a review of the 17 cases previously reported from other sites.

Among children and adolescents malignancies account for only 7–9% of orbital disease.[1,2] The most common primary malignant orbital neoplasm in children is rhabdomyosarcoma,[1,2] accounting for 26% of all orbital malignancies. Neuroblastomas are usually metastatic in children and are not commonly subjected to biopsy when they occur in the orbit because the primary site is already known. The reported incidence of orbital involvement derived from two pathology series is 1%,[1,2] which is probably an underestimate of the true clinical incidence.

Malignant ectomesenchymoma (MEM) is a rare and unusual tumour composed of both a mesenchymal element, most often rhabdomyosarcoma, and a neuroectodermal element, often neuroblastoma.[3,4] MEM are believed to arise from pluripotential migratory neural crest cells; they have been reported in the abdomen, perineum and scrotum, in the extremities, and in the middle ear, nasopharynx, face, and neck.

We report the first case of an orbital MEM, to our knowledge, with a review of the 17 cases previously reported from other sites.

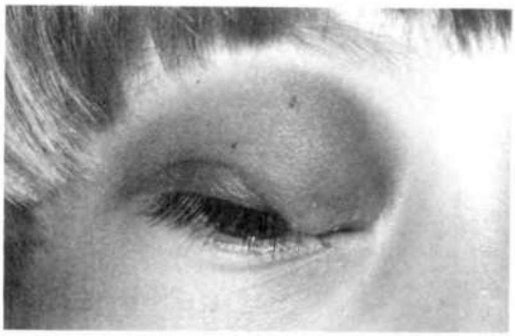

Figure 1 Ptosis induced by a right-sided superior nasal quadrant mass.

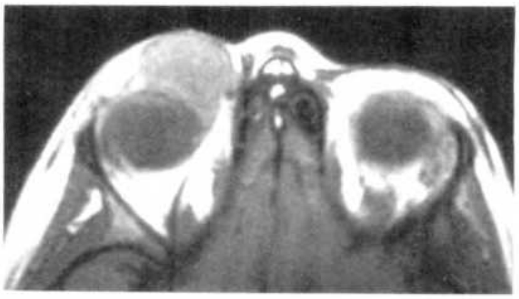

Figure 2A T1 weighted MRI demonstrates low signal intensity of the tumour.

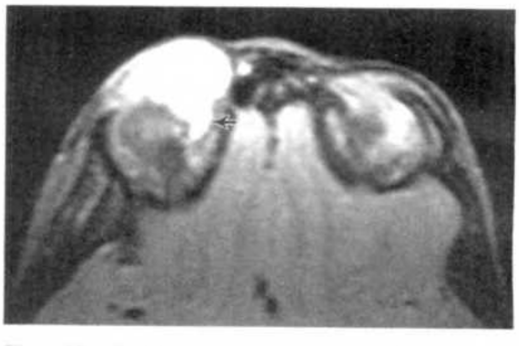

Figure 2B Gadolinium-enhanced T2 weighted MRI demonstrates enhancement of the mass. The tumour extends posteriorly in the superior nasal quadrant (arrow).

Case history

A 5½ year old girl was referred to the University of Washington Eye Center with a 10-day history of right ptosis and an enlarging mass in the superior nasal quadrant of the right orbit. The patient denied pain or any change in vision. The parents stated the mass had grown rapidly over the past week. Past medical history and family history were unremarkable.

The visual acuity was 20/50 on the right and 20/30 on the left. Right ptosis (7 mm) was noted, and the right globe was displaced 2 mm temporally and 3 mm inferiorly (Fig 1). There was no proptosis. Supraduction of the right eye was limited. A 1·5 cm firm mass was palpable protruding from under the right orbital rim in the superior nasal quadrant.

Magnetic resonance imaging showed a well demarcated 1·8 cm×2·2 cm×2·2 cm soft tissue mass in the right superior nasal orbit. The mass was primarily in the preseptal space but extended posteriorly over the globe in the nasal quadrant to the equatorial region (Fig 2). The sinuses and nasal cavity were normal.

A trans-septal anterior orbital biopsy was performed with debulking of the tumour.

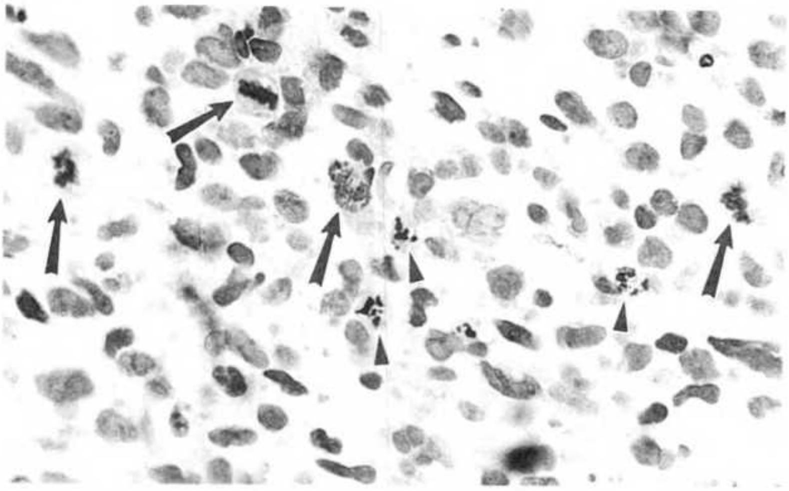

Figure 3 Loosely arranged round cells with scanty cytoplasm and hyperchromatic nuclei show marked pleomorphism with many mitotic figures (arrows). Some necrotic cells are present (arrowheads). (H-E, ×400.)

Grossly, the specimen consisted of a white, friable, minimally vascularised mass. The specimen was divided and processed by Sorvall JB-4 plastic embedment with haematoxylin and eosin stain for light microscopy and by standard techniques for electron microscopy.

HISTOPATHOLOGY

The tumour was composed of loosely arranged embryonal mesenchymal cells of varying sizes and shapes. Most cells were round with scanty cytoplasm and hyperchromatic nuclei and some necrotic cells were present (Fig 3). There were many mitotic figures (>5 per high power field), but no cross striations or rosettes were present (Fig 3).

Flow cytometry disclosed aneuploidy in 92% of the cells, with an average DNA content of 2·8 times normal and 23% of cells in the DNA synthesis phase (S phase; normal $<5\%$). The studies also indicated a small population of diploid cells.

Immunocytochemistry demonstrated two distinct, intermixed cell populations. One cell type was positive for desmin, vimentin, and muscle-specific actin. The other cell population was positive for chromogranin, nerve growth factor receptors, and neurofilaments. The specimen showed no reactivity with antibodies to lymphocytes, glial fibrillary acidic protein, carcinoembryonic antigen, or cytokeratins.

Electron microscopy demonstrated two cell populations, with regional segregation of cell types but some intermixing. One cell type consisted of closely apposed cells without cellular processes or basal laminae. These cells contained lipid and many mitochondria, as well as cytoplasmic filaments 5 nm and 15 nm diameter. Owing to the fixative used for electron microscopy, immunocytochemical confirmation that the 15 nm filaments were, in fact, myosin was not possible. No organised sarcomeres were identified, but foci of loose arrays of thick filaments were present in these cells (Fig 4). The other cell type included polygonal cells with numerous interdigitating cell processes. A few small intercellular junctions were present, but no desmosomes were found. These cells contained numerous mitochondria and 140–170 nm dense core neurosecretory-type granules (Fig 5).

CLINICAL EVALUATION

Liver function tests, chest *x* ray, chest and abdomen CT scans, cerebrospinal fluid cytology, liver-spleen scan, urinary catecholamines, and bone marrow were normal. The patient was treated according to the Intergroup Rhabdomyosarcoma Study III (IRS-III) regimen

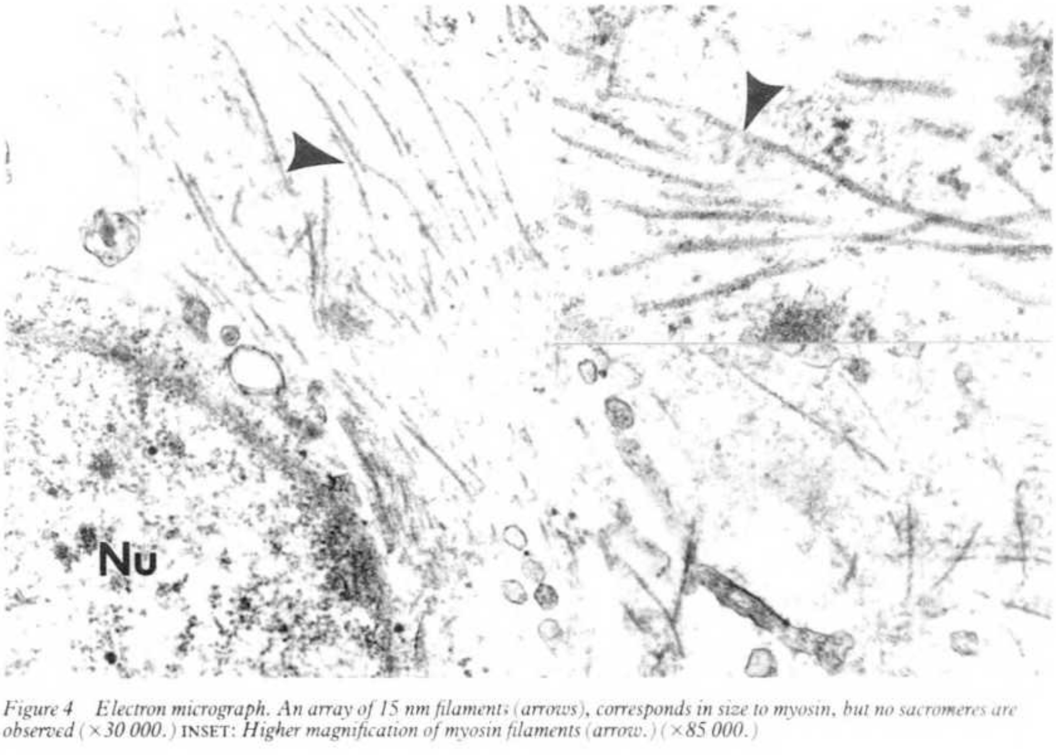

Figure 4 Electron micrograph. An array of 15 nm filaments (arrows), corresponds in size to myosin, but no sacromeres are observed (×30 000.) INSET: *Higher magnification of myosin filaments (arrow.) (×85 000.)*

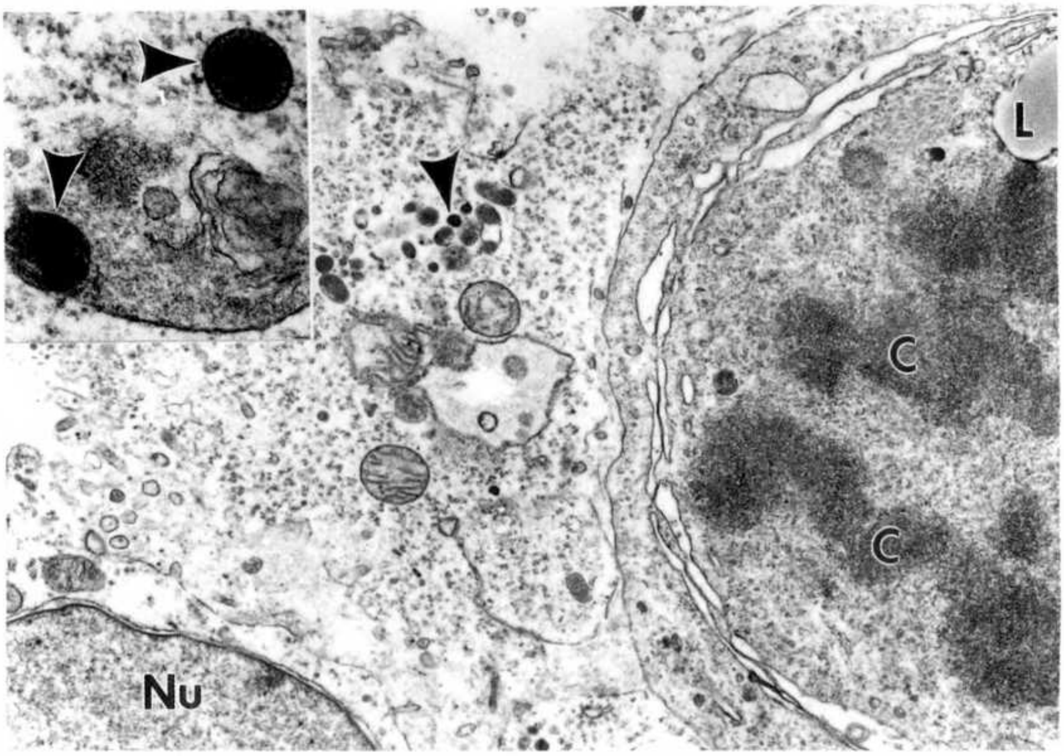

Figure 5 Electron micrograph. 140–170 nm dense-core neurosecretory granules are typical of neuroblastoma (arrows). (×8500.) C=dense chromosomes in a mitotic cell. L=cytoplasmic lipid droplet. INSET: *Higher magnification of dense-core granules (arrows). (85 000.)*

32 for rhabdomyosarcoma. Regimen 32 includes an initial course of 5 days of dactinomycin followed in 2 weeks by local radiation therapy and subsequent alternating courses of dactinomycin and vincristine. The patient's tumour grew rapidly during the dactinomycin treatment, so she was treated with 45 Gy of *x*-ray radiation in 1·8 Gy fractions to the tumour site beginning 10 days after initiation of chemotherapy. The tumour resolved, and the patient is well without recurrence or seconday tumour 18 months following completion of treatment. Follow-up magnetic resonance scans have not shown tumour regrowth. Her ptosis resolved and her vision improved to 20/25 on the right.

Discussion

Rhabdomyosarcoma is the most common primary orbital malignancy[1,2,5,6] and the third most common orbital lesion in children,[2] typically presenting as a rapidly growing orbital mass, most commonly in the superior orbit. One histological hallmark of rhabdomyosarcoma is the presence of cytoplasmic cross striations, representing attempted sarcomere formation, though cross striations are found in only 50–60% of tumours and are not required for correct diagnosis.[5,6] Neuroblastoma is the most common metastatic orbital tumour and the second most common orbital malignancy in children.[2] Two cases of primary orbital neuroblastoma have been reported in adults,[7,8] but none have been reported in children.

In the orbit, both rhabdomyoblasts and neural cells are believed to arise from pluripotential cells derived from migratory neural crest.[5,7,9] In the developing embryo almost all connective tissue elements in the globe and orbit arise from cranial neural crest cells, perhaps correlated with lack of paraxial somites in the head and neck region.[10–12] After migration into the orbit the neural crest cells undergo either mesenchymal or neuroectodermal differentiation, both of which are present in this tumour, which is accordingly termed 'ectomesenchymoma' or 'mesectodermoma'.

The tumour in this patient had intermixed mesenchymal and neuroectodermal components, as demonstrated by immunocytochemistry and electron microscopy. One type included cells that were positive for markers of skeletal muscle differentiation including desmin, vimentin, and muscle-specific actin. Although cross striations were not visible by light microscopy, electron microscopy demonstrated cytoplasmic filaments consistent with 15 nm myosin filaments. Myosin filaments occur primarily in striated muscle but are occasionally noted in smooth muscle cells. Myosin filaments in addition to coexpression of vimentin and desmin are diagnostic of rhabdomyosarcoma.[6] The other tumour component was composed of cells that were positive for markers of neuroendocrine differentiation, including chromogranin, neurofilaments, and nerve growth factor receptors. Dense core secretory granules (140–170 nm diameter) and intertwining cell processes were observed ultrastructurally, typical of neuroblastoma and aesthesioneuroblastoma. These neurosecretory granules were significantly smaller than granules found in other neuroectodermal tumours such as carcinoid and paraganglioma.[8]

The good general health of the patient and the normal results of physical, laboratory, and radiological examinations rule out metastatic neuroblastoma, aesthesioneuroblastoma, Ewing's sarcoma, and Wilms's tumour, and argue for the orbital primacy of this tumour. The fact that the two components of this patient's tumour were intermixed makes a so-called 'collision tumour' (two simultaneous primaries) less likely. In addition, flow cytometry demonstrated a single aneuploid population in this patient's tumour. Flow cytometry also showed that a high proportion of the tumour cells were in S-phase, consistent with the high mitotic rate observed by light microscopy.

MEM is a rare tumor, with 17 reported cases[3 4] plus this case of the first orbital MEM, to our knowledge. MEM usually presents in infancy (average age 17 months, range 0–66 months), but five cases have been reported in adults (average age 30 years, range 20–49 years). Various soft tissue sites have been involved: 10 cases occurred in the abdomen, perineum, or scrotum; two occurred in the extremities; six occurred in the head and neck region. Fifteen of the 18 cases contained rhabdomyosarcoma as the mesenchymal component and six of the 18 contained neuroblastoma as the neurosecretory component. At last follow-up eight patients (44%) were alive without tumour (mean of 38 months, range of 6–144 months), and six had died (mean of 14·5 months after presentation, range of 6–40 months). Four patients were lost to follow-up, one with known local recurrence and another with metastases. Tumour dissemination has occurred by direct extension, haematogenous spread to the lungs, intraperitoneal spread, and metastases to lymph nodes. MEM is a highly malignant tumour and should be treated aggressively.

Successful treatment of MEM had been achieved by resection and chemotherapy, with or without radiation therapy to the tumour site. Given that rhabdomyosarcoma is a frequent malignant component of these tumours, it has been suggested that a chemotherapy protocol recommended for rhabdomyosarcoma would be a logical approach.[3] Our patient was treated according to an IRS-III protocol. She responded well and is tumour-free with normal vision 18 months after presentation. Based on this case and reports in the literature, the appropriate treatment of MEM is maximal resection compatible with retention of function, combined with a rhabdomyosarcoma protocol of chemotherapy and radiation.

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Disc edge veins of Kraupa: rare exit anomalies of the retinal vein

Luiza Barroso, William F Hoyt, Michael Narahara

Neuro-Ophthalmology Unit, Departments of Ophthalmology, Neurological Surgery, and Neurology, University of California, San Francisco, California 94143, USA
L Barroso
W F Hoyt
M Narahara

Correspondence to:
William F Hoyt, MD,
Department of Neurological Surgery, c/o The Editorial Office, 1360 Ninth Avenue, Suite 210, San Francisco, California 94122, USA.

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Abstract

Disc edge veins of Kraupa are anomalies of the retinal venous system in which blood flows from the retina through a single venous trunk at or near the edge of the disc instead of at its centre. We report two examples of patients with these anomalies and illustrate the appearance of the anomalies with fundus photographs and a fluorescein angiogram. In one patient the retinal vein exited the eye through the sclera at the margin of the optic disc; in the other it disappeared into the disc tissue near the disc margin.

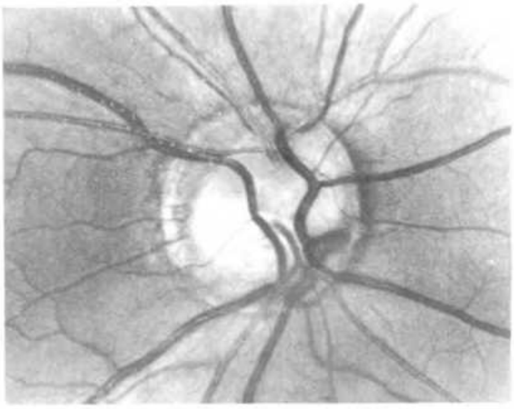

Figure 1 Disc edge vein of Kraupa in the right eye of the patient in case 1. The main trunk of the central retinal vein enters the bevelled scleral margin of the optic disc at 4 o'clock beneath the level of the pigment epithelium and choroid.

In 1915, Elschnig's assistant at the University Eye Clinic in Prague, Ernst Kraupa, published a drawing and description of a rare anomaly of the retinal venous system in which the main trunk of the retinal vein disappeared into the margin of the optic disc instead of its centre.[1] The first and only bilateral occurrence of this anomaly was recorded in 1916.[2] Kraupa added another example in 1924 in an extensive review and classification of arterial and venous anomalies at the optic disc margin. Altogether, we know of only four descriptions of Kraupa's

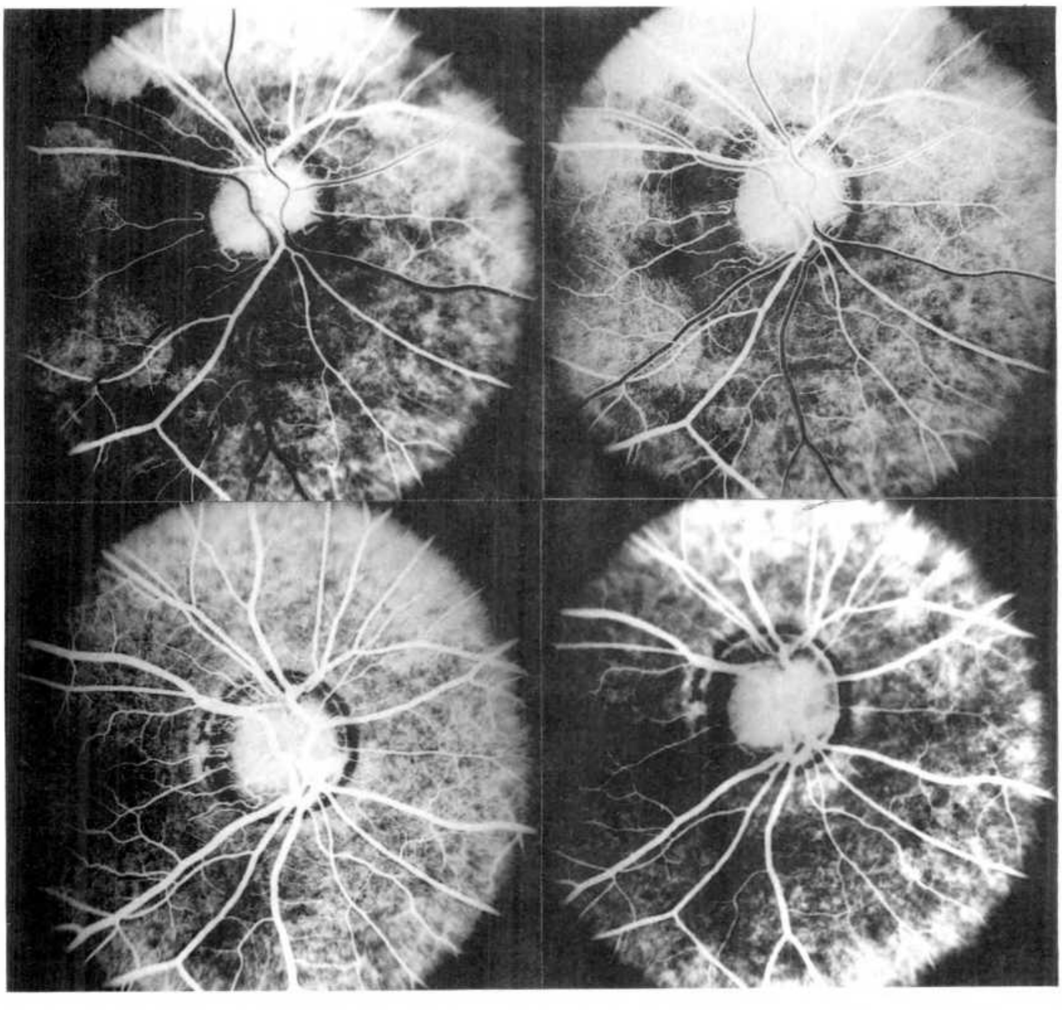

Figure 2 Fluorescein angiograms of the patient in case 1. Upper left: beginning laminar flow in the upper branches of the retinal vein at 17·9 seconds. Upper right: laminar flow in all retinal veins including the main trunk at 18·8 seconds. Lower left: complete fluorescein filling of the retinal veins at 25·3 seconds. Lower right: late venous phase at 162·2 seconds. Note absence of pooling of dye in the choroid around the exit point of the main venous trunk.

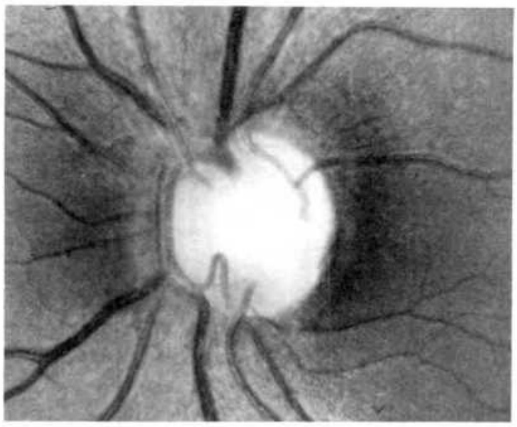

Figure 3 Disc edge vein of Kraupa in the left eye of the patient in case 2. The main trunk of the central retinal vein directly enters the optic disc at 11 o'clock. The retina is supplied by four arteries with separate exits at the nerve head. The central portion of the optic disc is devoid of arteries, veins, and optic cup.

disc edge vein, all occurring before 1949.[1-4] This report describes two differing examples of this anomaly, the first to be illustrated with modern fundus photographs and, in one case, fluorescein angiograms.

Case reports

CASE 1

A 25-year-old man who came to our eye clinic for a routine eye examination had mild myopia, normal corrected visual acuities, and normal visual fields. Both of his optic discs were small in diameter, had a bevelled scleral margin, and were flat without evidence of a physiological cup. The right disc had a striking anomaly of the retinal venous system in which all branches of the retinal vein joined in a common trunk that entered the bevelled disc margin inferonasally at 4 o'clock (Fig 1). Stereoscopic views confirmed that this venous trunk disappeared into the scleral edge beneath the level of the retinal pigment epithelium and choroid. The superior temporal vein crossed the disc surface and joined the inferior temporal vein above the disc margin at 6 o'clock without giving off a branch into the central portion of the disc. The central retinal artery issued from the disc as superior and inferior arterial trunks, which probably came from a single central arterial trunk that was invisible beneath the disc surface.

Fluorescein angiography (Fig 2) showed smooth and progressive laminar flow in the venous branches and the common venous trunk between 17·3 and 22·5 seconds. The venous tree was uniformly opacified with dye at 23·4 seconds. There was no pooling (increased concentration) of dye in the peripapillary choroid near the entrance of the common retinal venous trunk at any time. The dye column in the trunk vessel disappeared from view at the scleral margin. The choroidal filling was at first patchy but became complete between 18 and 25 seconds.

CASE 2

A 35-year-old woman was examined in our unit for evaluation of eye movement abnormalities associated with advanced cerebellar degenerative disease. Her visual acuities and visual fields were normal and she had no significant refractive error. Examination of both ocular fundi showed striking anomalies of retinal veins and arteries at the optic discs. The discs were slightly smaller than normal in diameter, had a flat cupless surface, and had no major vessels entering or leaving the central region.

The right disc had four arteries with separate exits at the nerve head, each artery supplying one of the quadrants of the retina. No central retinal artery was seen. The retinal veins formed superior and inferior venous trunks that converged above and below the disc and then entered the disc tissue, probably joining as a central venous trunk beneath the disc surface. The left disc (Fig 3) also had four arteries with separate exits at the nerve head, each supplying a retinal quadrant, and there was no central retinal artery. All retinal venous branches converged into a common venous trunk that ran upward over the nasal disc margin and entered the disc tissue inside the disc margin at 11 o'clock.

Discussion

Our cases conform closely with Kraupa's descriptions of disc edge veins draining the retina through a single trunk (Optikusrandvene). The veins in our two cases differ from each other in the way they exit the eye. In case 1 the vein enters the wall of the scleral canal, whereas in case 2 it enters the nerve itself. Our examination of published drawings of disc edge veins showed similar differences. Kraupa's first case[1] and von Herrenschwand's bilateral case[2] resemble our case 1; Kraupa's second case[3] and Oxilia's case[4] resemble our case 2.

There is no published report of a histologically studied disc edge vein. Therefore, the various paths taken by such veins after they disappear from ophthalmoscopic view and pass into the orbital venous plexus remain a matter for speculation. Our two cases show clearly that disc edge veins can take at least two routes out of the eye.

Elschnig[5] stressed the extreme rarity of disc margin arteries occurring in conjunction with disc margin veins. Our case 2 is noteworthy in this regard. It is an example in which multiple disc margin arteries supplying all quadrants of the retina were present in a patient who also had a Kraupa's disc edge vein. Lawford[6] illustrated a similar anomaly in 1895.

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British Journal of Ophthalmology, 1992, 76, 444–446

Recurrent malignant melanoma of the corneal stroma: a case of ‘black cornea’

A D A Paridaens, C M Kirkness, A Garner, J L Hungerford

Abstract

A 39-year-old Caucasian woman with a history of recurrent conjunctival melanoma of her right eye developed an intrastromal heavily pigmented malignant melanoma, which involved the whole corneal diameter. The patient was treated by corneoscleral lamellar keratoplasty and there has been no evidence of recurrent neoplasm during 4 years of follow-up. This apparently unique presentation of malignant melanoma of the cornea is illustrated and the differential diagnosis of corneal pigmentation is discussed.

Malignant melanoma of the cornea is very uncommon. Though primary corneal melanoma has been reported[1–5] this neoplasm is usually seen in association with an adjacent limbal or conjunctival melanocytic lesion. We describe a patient with a history of recurrent conjunctival melanoma who presented with a black cornea due to extensive malignant melanocytic invasion into corneal stroma.

Case report

In February 1987 a 39-year-old Caucasian woman was referred to Moorfields Eye Hospital with a 9-year history of recurrent malignant melanoma of her right conjunctiva. The diagnosis had been established by excision biopsies of temporal perilimbal pigmented tumours in 1983 and 1985, which showed that the melanoma originated from an area of primary acquired melanosis with atypia. No additional cryotherapy or β irradiation had been given.

She complained of having had right photophobia and blurred vision for several months. On examination there was extensive and dense pigmentation of the entire cornea, interfering with the central visual axis (Fig 1A). Slit-lamp examination showed involvement of superficial corneal stroma. There was evidence of melanosis of the temporal limbus but no nodular disease was noted. No further areas of conjunctival pigmentation were seen. The right visual acuity was 6/18 with pinhole and 6/36 unaided. The left unaided visual acuity was 6/6. The intraocular pressure was 10 mm Hg in both eyes. The left eye showed no abnormalities.

In June 1987 a corneoscleral lamellar keratoplasty was performed. More than two thirds of the thickness of the whole cornea were excised en bloc with a scleral component in the inferotemporal quadrant, which resulted in a keyhole shaped defect with a diameter of 15 mm by 24 mm (Fig 2). In addition diagnostic biopsies were taken from nasal conjunctiva, Tenon's capsule, and inferior fornix. A donor corneal graft was then sutured to the edges of the corneal host defect using interrupted 10/0 Nylon. The conjunctiva was closed over the bare scleral component of the donor corneal graft, so that the graft was covered to the level of the host limbus.

Postoperatively, there was a mild corneal stromal haze and the epithelial defect of the donor graft healed within 2 weeks. Some pigment and interface irregularity was noted at the graft host interface at 10 o'clock and both superficial and deep neovascularisation were seen at 6 o'clock. One year after the surgery, the cosmetic result was very satisfactory (Fig 1B) and no noticeable changes of the pigmentation and vascularisation were observed, except for a small area of flat pigmentation of the corneal edge at the 10–11 o'clock position. The best corrected visual acuity was 6/9 (20/30) with −5 D. Because of anisekonia the patient was fitted with a daily wear soft contact lens, which was well tolerated. Topical steroid therapy has been continued in gradually decreasing dosage (currently prednisolone drops, 3% twice daily).

On her last visit in May 1991, 4 years after removal of the corneal malignant melanoma, the visual acuity was 6/9 and there was no evidence of recurrence of frank malignancy.

HISTOPATHOLOGY

Histopathological examination of sections of the cornea and adjacent sclera showed a small focus of residual or recurrent malignant melanoma at the periphery of the cornea within the superficial stroma (Figs 3 and 4). Heavily pigmented cells were observed between stromal lamellae across the entire corneal diameter, almost reaching the resection margin on the opposite side. The corneal epithelium was atrophic but otherwise normal and there was no appreciable stromal vascularisation. Some infiltration of the adjacent bulbar conjunctiva by neoplastic cells was also seen together with mild inflammation in the underlying stroma. Within the conjunctiva the tumour was intraepithelial. Separate specimens from the vicinity of the lateral rectus muscle, the inferior Tenon's capsule, and from the nasal conjunctiva showed no abnormality.

Discussion

Pigmentation of the cornea is grossly or biomicroscopically visible and is of several origins including ocular inflammation, conjunctival melanocytic lesions, anterior segment trauma, the pigment dispersion syndrome,[6] and drugs.[7,8] Depositions of metals, blood pigments, lipids, and lipofuscin[9] may mimic the gold-brown to black melanin pigmentation.

Institute of Ophthalmology, Department of Pathology
A D A Paridaens
A Garner

Moorfields Eye Hospital, Cornea Clinic
C M Kirkness*

Moorfields Eye Hospital, Oncology Clinic
J L Hungerford

*Current address:
Tennent Institute of Ophthalmology,
University of Glasgow,
Glasgow

Correspondence to:
Dr A D A Paridaens, Institute of Ophthalmology,
Department of Pathology,
17/25 Cayton Street,
London EC1V 9AT.

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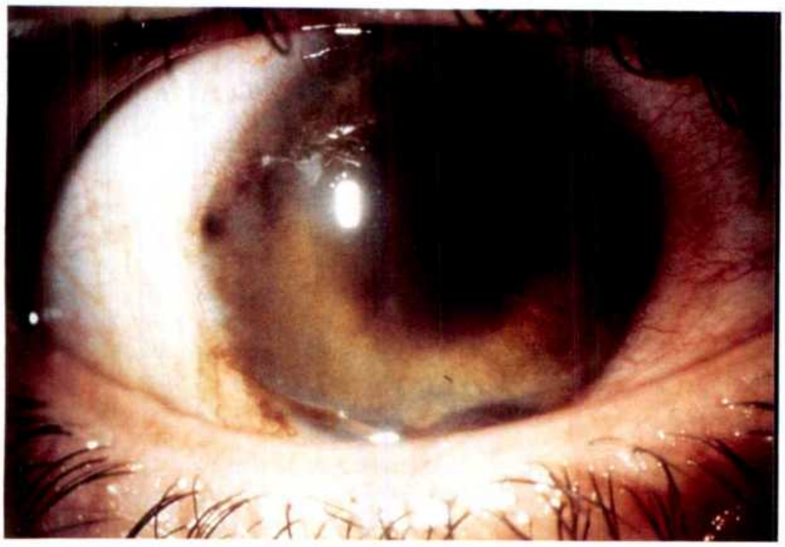

Fig 1A

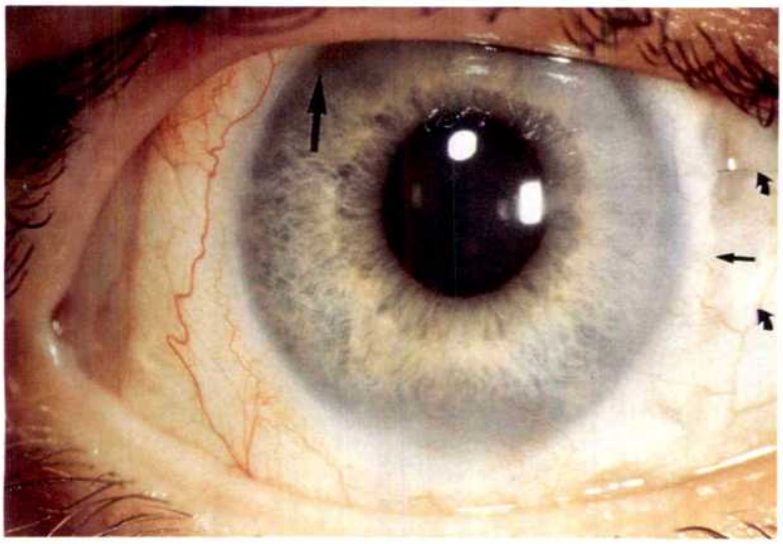

Fig 1B

Figure 1 (A) 'Black cornea': extensive and dense pigmentation of the entire right cornea of a 39-year-old woman with a 9-year history of recurrent conjunctival malignant melanoma of her right eye. (B) One year after lamellar keratoplasty, the cosmetic result is very satisfactory. Except for a small area of flat corneal pigmentation (big arrow) between the 10 and 11 o'clock meridian, no pigmentary changes are noted. The edge of the lamellar graft (small arrow) and some stitches (curved arrows) can be identified.

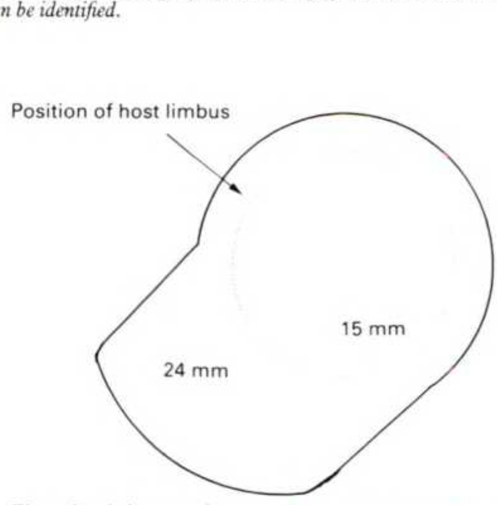

Figure 2 A donor graft was used to cover the keyhole shaped defect with a diameter of 15 mm by 24 mm, which was the result of en bloc excision of more than two thirds of thickness of the entire cornea with a scleral component in the inferotemporal quadrant.

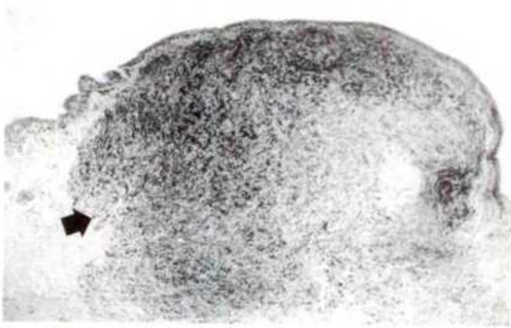

Fig 3A

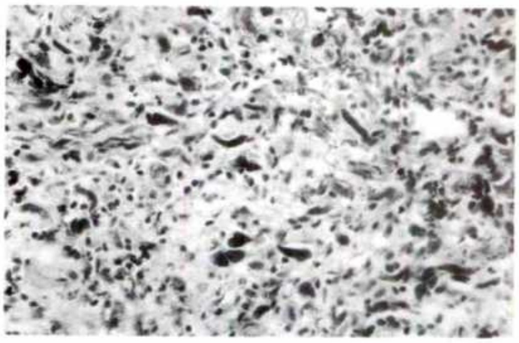

Fig 3B

Figure 3 (A) Histological examination of biopsy specimens of the right temporal limbus and conjunctiva shows conjunctival malignant melanoma (arrow) originating from areas of primary acquired melanosis with atypia (haematoxylin and eosin, ×20). The maximal thickness of this nodule is 1·8 mm. (B) Higher power examination shows heavily pigmented, large pleomorphic cells and spindle cells (haematoxylin and eosin, ×200).

The location of corneal pigmentation depends on the underlying cause. Posterior corneal melanin pigmentation has been described in association with congenital melanosis oculi,[10] iris melanocytic proliferation,[11] endothelial phagocytosis of free melanin pigment, and the presence of melanophages or iris pigment epithelial cells.[12] Melanin pigmentation of the anterior cornea has been shown to increase with age and to be related to melanosis of the bulbar conjunctiva.[13] Other causes include the presence of melanin in Langerhans cells, melanophages, neoplastic squamous cells, or in the extracellular space.[14]

Healthy corneal tissue is transparent and does not harbour melanocytes, with the exception of the corneal limbus. Limbal melanocytes may migrate into the corneal epithelium upon exposure to sunlight or chemicals,[15] and both adjacent malignant melanosis and frank melanoma may spread locally to the cornea. When the population of atypical melanocytes is confined to the epithelium the condition is defined as 'corneal primary acquired melanosis with atypia'; when there is invasion of Bowman's layer and stroma of the cornea the lesion is by definition a 'corneal malignant melanoma'. Primary corneal melanoma[1-5] may be unassociated with limbal or conjunctival neoplasia, but is thought to originate from melanocytes, which have migrated from limbus or conjunctiva. A corneal malignant melanoma may vary in its appearance from nodular and amelanotic[1 3] to flat and pigmented as in our case.

Melanocytic invasion complicating conjunctival melanoma rarely occupies more than one quadrant of the cornea. The presentation of recurrent disease as described in the above case report was unique in our experience and has in our knowledge not been reported before. Histopathological examination showed absence of atypical melanocytes in the corneal epithelium or between Bowman's layer and the basal membrane. The direct spread of melanocytes into the corneal stroma may have been assisted by previous excisions of limbal melanomas that were removed with an en bloc lamellar dissection of the involved cornea and sclera. The inability of damaged Bowman's membrane to regenerate results in a defective barrier to cellular invasion. The compact lamellar architecture of the cornea, which limits both intraocular tumour spread and its associated blood supply, may underly the relatively good prognosis for corneal malignant melanomas.

Fig 4A

Figure 4 (A) A small focus of residual or recurrent malignant melanoma (big arrow) is seen at the periphery of the cornea within the superficial stroma. Tumour cells are present in the corneal stroma (small arrow) but the corneal epithelium (curved arrow) shows no evidence of melanocytic infiltration (haematoxylin and eosin, ×120). (B) Pigmented atypical melanocytes (arrows) have invaded the cornea between the stromal lamellae. The melanocytic invasion involves more than half the corneal thickness and the entire corneal diameter to almost reach the resection margin on the medial side (haematoxylin and eosin, ×320).

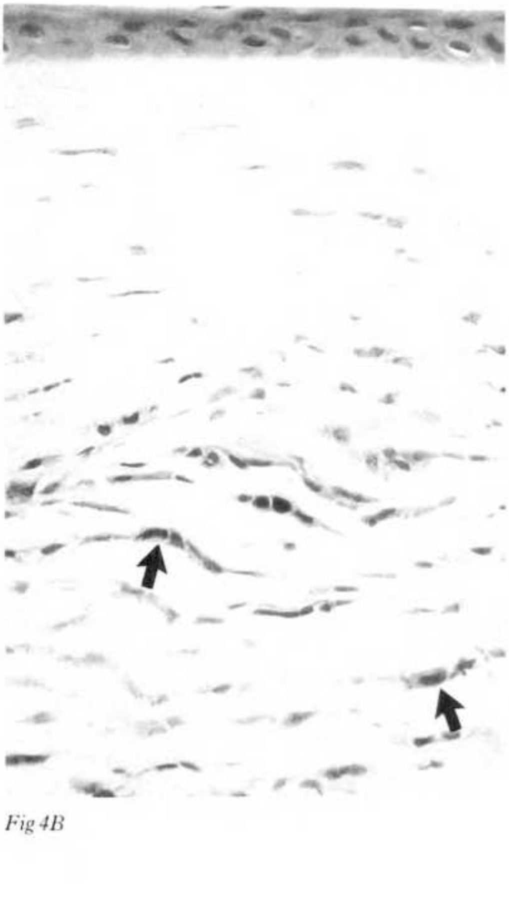

Fig 4B

There may be several approaches to the treatment of corneal melanoma or primary acquired melanosis. Melanocytic tumours that are confined to the corneal epithelium may be treated by sliding off the epithelium using absolute alcohol. Lesions involving the superficial corneal stroma may be treated by either superficial keratectomy and freeze-thaw cryotherapy or lamellar keratoplasty. Deeper stromal tumours may be managed by penetrating keratoplasty.[16] A more radical approach in the management of invasive corneal melanoma secondary to primary conjunctival disease is exenteration of the orbit. In our case the relatively young woman, who was treated conservatively, has not only been able to keep her eye, which is cosmetically excellent, but also she has essentially normal visual acuity. Furthermore, in the 4 years of follow-up, no signs of local or distant recurrence of her tumour have been noted.

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LETTERS TO THE EDITOR

Ocular leprosy

SIR,—I very much enjoyed the recent mini review on leprosy by Dr T J ffytche.[1] It is always interesting for those of us working in what are sometimes regarded as exotic or obscure specialties to see our interests featured in other journals and it does, of course, bear repeating that such apparent exotica appear from time to time among the more mundane workload of western developed medicine.[2] Hence your journal's recent articles featuring ocular leprosy are an important contribution to maintaining a level of necessary awareness of this problem among your regular readers both abroad and at home.

There were, however, some points in the review which merit comment. It is, of course, accepted that motor and sensory disturbances involving the eye are found throughout the spectrum of leprosy.[3] While loss of lid function caused by weakness or paralysis of the orbicularis oculi muscle (lagophthalmos) is more often found in tuberculoid, borderline tuberculoid, and borderline patients, under the Ridley-Jopling classification,[4] as a result of a type I (reversal) reaction involving the zygomatic branch of the facial nerve, this does not usually coincide with significant corneal hypoaesthesia, which is much less common.[5] While a degree of corneal sensory loss can be detected in some patients with lagophthalmos of several years' duration, sensory loss on the cornea is more a feature of longstanding borderline lepromatous disease, and lepromatous disease where bacillary infiltration is more likely to be found in the ciliary nerve endings running towards the limbus and cornea and where there may have been repeated episodes of iritis.[6] The inflammatory process in the facial nerve is the result of a sudden increase in specific cell-mediated immunity against the leprosy bacilli which concentrate in the cooler part of the nerve as it crosses the zygoma. The motor function loss is therefore almost invariably of sudden onset and does not take place over a long period of time. The patient may, however, take a considerable time to report this new nerve damage. The treatment of lagophthalmos of less than 6 months' duration is systemic corticosteroids. The other anti-inflammatory drugs mentioned in the mini review, clofazimine and thalidomide, have no place in the management of leprosy-induced facial palsy. On the contrary, it is for repeated iridocyclitis, a result of type II (erythema nodosum leprosum or ENL) reaction, that these two drugs can be so valuable.[7] This is sometimes complicated in borderline lepromatous patients who can develop both types of reaction, occasionally one triggering off the other, and hence the picture is not always as clear as the textbooks would have us believe.

Despite this, facial patches in tuberculoid, borderline tuberculoid, and borderline leprosy patients do not go into type II or ENL reaction, implied in the mini review. It is the type I reaction in a facial patch which is such a sinister presentiment for the integrity of the facial nerve and this was clearly described by Hogeweg *et al* in their recent paper.[8]

RICHARD DE SOLDENHOFF
*Netherlands Leprosy Relief Association,
Eastern Leprosy Control Programme,
Nepal*

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⁂(This letter arrived after Mr ffytche's reply to Dr Hogeweg, *Br J Ophthalmol* 1992; **76:** 254–5.

Use of a blue filter in visual field analysis

SIR,—As an alternative to blue-on-yellow perimetry for more sensitive visual field testing in glaucoma, Hugkulstone and Vernon[1] recently proposed blue-on-blue perimetry (usage of blue glasses in combination with a white-on-white perimeter). Technically this would be an attractive alternative, but is this alternative equivalent to blue-on-yellow perimetry?

The concept of blue-on-yellow perimetry is based on the so called two-colour threshold method introduced by Stiles in the late 1940s.[2] Application in the present field was discussed by Johnson *et al.*[3] Stiles monitored the threshold of a test stimulus as a function of the luminance of a background. In certain wavelength combinations, different branches in the threshold function could be distinguished, indicating response systems with different spectral sensitivities. Stiles called these systems pi mechanisms. They represent the functional channels of the visual system, with characteristic spectral sensitivities in three wavelength regions. Pi-1 to pi-3 resemble blue cone (short wavelength sensitive (SWS)) sensitivity. Pi-4 green cone (medium wavelength sensitive (MWS)) sensitivity and pi-5 red cone (long wavelength sensitive (LWS)) sensitivity.

From the two-colour threshold theory can be calculated that in routine perimetry, stimulus and background conditions (because of their luminance and spectral composition) are such that MWS and LWS channels are tested. Blue-on-yellow perimetry is designed to test the SWS channel selectively. Figure 1 shows the spectral sensitivity of the three systems for no background. The SWS channel has its maximum sensitivity at 440 nm (blue). The MWS and LWS channels however have the same sensitivity at this wavelength as the SWS channel. In order to test the SWS channel selectively it is necessary to decrease the sensitivity of the MWS and LWS channels, relative to the sensitivity of the SWS channel. This is achieved by a yellow background that contains only wavelengths above 550 nm. Maximum separation between the sensitivity of the SWS channel and the MWS and LWS channels is reached if the brightness is 200 cd/m^2.[4] It can be derived from the two-colour threshold theory that the separation reached at 440 nm is about 2·5 log units (25 dB) (Fig 2). Stiles' theory was based on foveal vision. Johnson showed a comparable separation to be present in peripheral vision.[5] Unless these conditions are met it is not possible to test the SWS channel selectively.

What is to be expected if blue stimuli are presented on a blue background? This background decreases the sensitivity of the SWS channel, but also of the MWS and LWS channels. At 440 nm the relative position between the SWS channel and the other two channels is about the same as in Figure 1. So the blue stimulus is again perceived by all three channels. This might explain why the authors did not find significant differences between the blue field score as compared to the standard condition. If a blue stimulus on a white background is used a separation of only about 0·7 log units (7 dB) results, but this seems to give no advantage above a white stimulus on a white background.[6] In both conditions the MWS and LWS channels may play an important role.

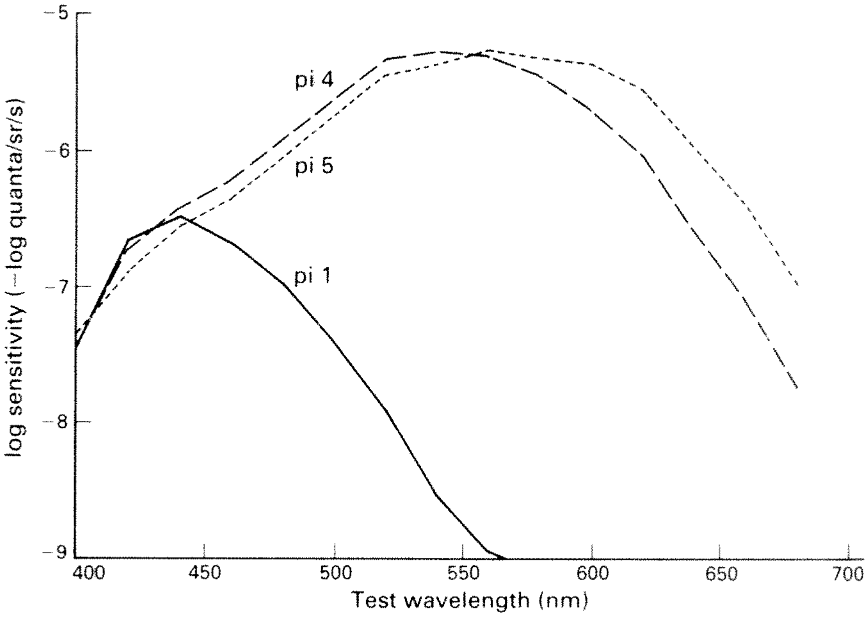

Figure 1 Spectral sensitivities of the pi mechanisms for the no background conditions.

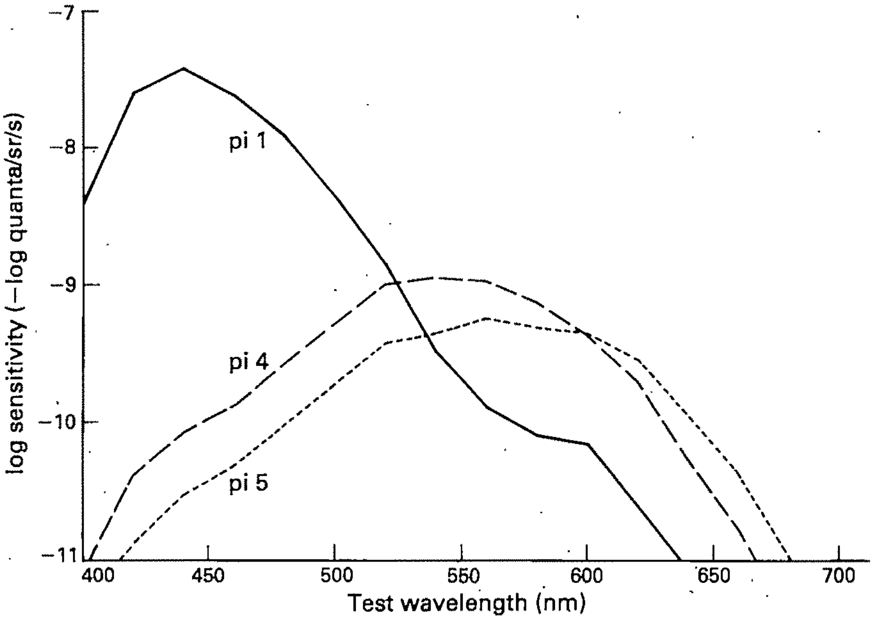

Figure 2 Spectral sensitivities of the pi mechanisms if a yellow background (Schott OG 550) with a brightness of 200 cd/m² is used.

The transmission of blue light is much more dependent on the amount of lens opacification than the white light. This might have influenced the blue field score. The absorbance of the blue light can vary considerably above the age of 50 years, depending on the lens coloration of each individual.[5] This variability of lens absorbance remains even if groups are age matched. This puts a serious limitation on the application of tests that measure the general level of blue sensitivity (colour vision, anomaloscopy, blue field score) without correction for the blue light absorbance in the ocular media.

This problem was overcome[1] by use of the selective blue field (SBF) score. However it was not made possible to judge whether this score offers an advantage since this score was not compared with a similar score using white-on-white perimetry.

The SWS channel is said to be more prone to glaucomatous damage.[3] This however should not erroneously be coupled[1] to the findings of Quigley[7] that the larger than average ganglion cells are more prone to glaucomatous damage. Although the ganglion cells that receive input from the SWS cones are probably larger than the ganglion cells that receive input from the MWS and LWS cones, the ganglion cells that process colour vision belong to the smaller than average group of ganglion cells.[8] Alternative explanations have been proposed.[9]

The finding of Hugkulstone and Vernon[1] that wearing blue glasses during perimetry is a more sensitive method to detect early glaucomatous damage of course awaits validation by other researchers. If their conclusion would prove to be true it should not be coupled to the findings in blue-on-yellow perimetry, because this technique is not based on the isolation of the SWS channel.

L A M S DE JONG*
J FELIUS†
T J T P VAN DEN BERG*,‡
E L GREVE†
**Netherlands Ophthalmic Research Institute*
†Glaucoma Centre University of Amsterdam
‡Department of Medical Physics and Informatics,
University of Amsterdam

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Reply

SIR,—We are pleased that Drs de Jong, Felius, van den Berg and Greve have shown an interest in our paper.

Table 1 Mean (range) white and selective blue field (SBF) scores

	Normal	*Glaucoma*
White field score	0·5 (0–8)	14·5 (0–72)
SBF score	7·5 (0–25)	28·4 (0–66)

To answer one of their later points, the mean (range) selective blue field (SBF) scores are compared with a similar scoring system applied to the white field for normals and patients with early glaucoma in Table I. The SBF score shows a marked increase over the selective white field score in the early glaucoma group, despite the fact that the white field was used as one of the defining characteristics for the groups.

We agree that our technique of using a blue filter is not as precise as the use of a blue stimulus on a yellow background, and therefore does not totally isolate the response of the short wave sensitive (SWS) cones. However, we feel that as our protocol increases the contribution of the SWS cones to the field score, it will increase the size and depth of glaucomatous defects, as was originally described by Genio and Friedmann.[1]

Interestingly, our findings support Genio and Friedmann,[1] whereas Logan and Anderson[2] contradicted them. Both of these studies used the blue filter on the standard background of a Friedmann Visual Field Analyser, thus altering the Weber-Fechner relation whereas our use of a spectacle-mounted filter preserves this relation. However, our results support one study but refute another similar one – an interesting anomaly. It is possible that the degree of the defect played a role, as it has been suggested that even blue on yellow perimetry is no more sensitive than standard testing for manifest defects.[3] Also, we used the threshold determined for a white stimulus whereas both Genio and Friedmann[1] and Logan and Anderson[2] determined the threshold setting for a blue stimulus.

Finally, we agree entirely that our findings await confirmation by others. We are currently investigating the use of blue filters with automated threshold perimetry, controlling for lens density.

CHARLES E HUGKULSTONE
STEPHEN A VERNON
Academic Unit in Ophthalmology,
University Hospital,
Queen's Medical Centre,
Nottingham NG7 2UH

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NOTES

The Vitreoretinal Frontier

A meeting entitled 'The Vitreoretinal Frontier' will be held on 6–7 November 1992 in Dallas, Texas. The course directors are R Joseph Olk, MD and William B Snyder, MD. Further details: Jeanne B Toma, 8816 Manchester Road, Suite 288, St Louis, MO 63144, USA. (Tel: (314) 968-1961; Fax: (314) 968-3066.)

International Conference on the Lacrimal Gland, Tear Film and Dry Eye Syndromes

From 14 to 17 November 1992, the International Conference on the Lacrimal Gland, Tear Film and Dry Eye Syndromes: Basic Science and Clinical Relevance, will be held in the Southampton Princess Resort in Bermuda. This meeting will address the current knowledge and 'state of the art' research on the structure and function of lacrimal tissue and tears in both health and disease. For additional information, please contact: Dr David A Sullivan, Immunology Unit, Eye Research Institute, 20 Staniford Street, Boston, MA 02114, USA.

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BRITISH JOURNAL OF OPHTHALMOLOGY

Editorial

Recent advances in scientific methods and techniques have produced marvellous insights into normal physiology and also into pathophysiological mechanisms of disease. Take for example, the remarkable molecular genetic studies linking resistance/susceptibility to diabetes mellitus to the presence or absence of the amino acid aspartic acid at position 57 in that region of the MHC Class I molecule which is involved in antigen presentation.[1] Or consider the explosion of knowledge in cell growth and proliferation: mitosis (and meiosis) it appears, are orchestrated by a group of proteins known as cyclins, in a very precise manner[2] and this has direct bearing not only on our understanding of neoplasia but also on potential anticancer therapeutic strategies. The development of monoclonal antibody technology has advanced well beyond immunodiagnostics and is rapidly invading clinical therapeutic practice (using methods which involve targeting specific antigens).

Ophthalmology has not been untouched by these advances. The molecular genetics of retinitis pigmentosa has identified a range of specific genetic defects in a series of photoreceptor proteins,[3] and some sophisticated research has revealed the elements of spectral tuning in cone photoreceptors by various cone opsins.[4] In the clinic, PCR (polymerase chain reaction) technology is not simply a research tool but is being applied widely to many ophthalmic (and non-ophthalmic) diagnostic problems and may prove very valuable in early diagnosis of infection. In addition, rapid advances in imaging techniques are having a direct influence on the practice of ophthalmology.

How does the ophthalmic community become informed of developments in scientific methods so as to take advantage of them? Conference proceedings and research papers are the major vehicles for dissemination of information, and the *British Journal of Ophthalmology* participates in this process. Recent changes in the editorial organisation have afforded the opportunity to facilitate this function of the journal and the proposed modifications to its content described below are aimed in this direction.

In the coming months, the journal plans to introduce a section on laboratory sciences which will reflect a growing interest in investigative ophthalmology. Accordingly, articles in this field will be welcomed, but not to the detriment of original articles in clinical ophthalmology, which will continue to provide the foundation of the journal's published material. Review articles which outline the relevance of and links between the laboratory, the experiment, and the clinic will be particularly welcomed. The emphasis on single case reports will be reduced and a new format for the case reports instituted. To balance this, a new feature will be introduced providing a brief commentary on clinical practice points.

Not all of these changes can be introduced at once and specific instructions as to the preparation of the different articles require to be formulated. These will appear in coming issues. However, these are scientifically exciting times and ophthalmology has much to give to the wider medical scientific community. We trust that our readers agree that a restatement of the original aims of the journal to cover both clinical and laboratory visual and ophthalmic sciences is timely and appropriate.

J V FORRESTER

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British Journal of Ophthalmology 1992; 76: 450–452

ORIGINAL ARTICLES

Inhibition of intraocular fibrin formation with annexin V

P Chollet, F Malecaze, F Hullin, P Raynal, J L Arne, V Pagot, J Ragab-Thomas, H Chap

Laboratory of Ophthalmology, Hôpital de Rangueil, Toulouse, France
P Chollet
F Malecaze
J L Arne
V Pagot

Unit 326 of INSERM (National Institute for Research in Health and Medicine)
F Hullin
R Raynal
J Ragab-Thomas
H Chap

Correspondence to:
Dr P Chollet, Laboratory of Ophthalmology, Hôpital de Rangueil, 1 avenue Jean Poulhès, 31054 Toulouse, France.

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Abstract

Annexin V is a member of the calcium- and phospholipid-binding proteins, known to have an antithrombotic effect. For the first time, we have tested its ability to prevent intraocular postoperative fibrin formation in a standardised rabbit model and compared its effect with that of heparin. Annexin V, 20 µg and 60 µg, injected in the anterior chamber postoperatively, significantly reduced the area of the fibrin clot and its time to clearing. Annexin V appeared to be as efficient as heparin. It probably acts by preventing phospholipids from playing their role in the coagulation cascade which leads to fibrin formation. Furthermore, annexin V has an anti-inflammatory effect by protecting phospholipids from phospholipase A_2 activity. Therefore, annexin V might be considered as a new therapeutic agent acting both on fibrin formation and inflammatory processes.

(*Br J Ophthalmol* 1992; 76: 450–452)

After intraocular surgery, a more or less intense fibrinous reaction can occur. This intense fibrinous reaction prevents adequate postoperative examination and may lead to pupillary block glaucoma. Fibrin results from the extravasation of activated coagulation factors due to disruption of the postoperative blood aqueous barrier. An attempt to lessen its occurrence usually involves the use of topical or even systemic steroids which would theoretically act by stabilising vascular membranes; on the other hand, heparin is well known to inhibit coagulation and therefore fibrin formation. Johnson *et al*[1] have already shown its ability to prevent intraocular fibrin formation after surgery. However its use is not without complication in patients since it may enhance the frequency of intraocular bleeding.[2]

Annexin V is one of the seven members of the newly isolated annexin family. All of these proteins share the ability to bind to phospholipids in a calcium-dependent manner.[3,4] The precise function of annexin V remains uncertain, but it has been shown to have two main biological properties, resulting from this biochemical feature: an anti-inflammatory effect through inhibition of phospholipase A_2[4] and an antithrombotic effect through inhibition of the coagulation cascade, whose activation leads to fibrin formation.[5]

In this study we investigated the effectiveness of intracameral annexin V in preventing fibrin formation in a standardised rabbit surgical model and compared it with that of intracameral heparin.

Materials and methods

New Zealand white rabbits weighing 2·0 to 2·5 kg were used in this study. All animals were treated according to the Association for Research in Vision and Ophthalmology resolution on the use of animals in research. Rabbits were anaesthetised with an intramuscular injection of 50 mg ketamine. Surgical procedures were according to Johnson *et al*[1] with few modifications: a sclerotomy was made 1 mm posterior to the limbus and a central vitrectomy was then performed with lactated Ringer's as posterior infusion solution. No lensectomy was performed since lens persistence is considered to localise the fibrin clot to the anterior chamber and therefore to facilitate the estimation of postoperative fibrin reaction.[1] Five minutes later, cyclocryotherapy was performed. A cryoprobe was placed at the limbus at the 3, 6, 9, 12 o'clock positions and freezing was performed for 45 seconds at −80°C. An anterior chamber puncture (100 µl) was then made, followed by an intracameral injection of annexin V, heparin, or Ringer's lactated solution: 18 rabbits underwent an injection of annexin V: 5 µg (n=6), 20 µg (n=6), or 60 µg (n=6). A second group (n=18), the controls, was injected with lactated Ringer's solution.

Six rabbits were operated simultaneously on both eyes. At the end of surgery, one eye was injected with 20 µg of annexin V, the other one with 15 IU heparin.

All injections were diluted with lactated Ringer's solution so that the final volume was 100 µl. Care was taken to avoid contact with any structure of the anterior chamber. No antibiotic or anti-inflammatory treatment was used postoperatively.

Rabbits were then examined daily: after an instillation of topical anaesthesic, the anterior chamber was examined with a surgical microscope and was assessed for the presence or absence of fibrin. Fibrin tended a form a contiguous white amorphous clot or, in less obvious reactions, led to a decrease in the fundus red reflex or an obscuration of the iris details. Fibrin clot reaction was evaluated in a similar manner to that used by Johnson *et al.*[1]

Two parameters were taken into consideration: (1) *Fibrin clot area on the first postoperative day.* The anterior chamber was divided into four quadrants. Fibrin clot was graded on a scale of 1+ to 4+. A 1+ clot involved an area up to one quadrant; a 2+ clot involved an area up to two quadrants: a 3+ clot involved an area up to three quadrants, and a 4+ clot involved an area up to four quadrants.

Light fibrin reactions, obscuring iris details, with no 'real' clot, were recorded as grade 1+.

(2) *The time until clearing of the fibrin clot.* It was recorded as the first postoperative day on which no more fibrin was to be seen. There were two examiners for all experiments, which were performed in a double-blind manner.

The annexin V used in this study was purified in our laboratory from human placenta, according to the procedure described by De *et al.*[6]

To evaluate the effectiveness of annexin V in reducing postoperative intraocular fibrin, we used the Mann-Whitney *U* test and the Wilcoxon-signed rank test to compare its efficacy with that of heparin.

Results

Surgical procedure resulted, in all cases, in a fibrinous reaction on the first postoperative day.

EFFECTIVENESS OF ANNEXIN V IN INHIBITION OF POSTOPERATIVE FIBRIN CLOT

Annexin V-injected eyes, at doses of more than 5 μg, had less fibrinous reaction and a shorter time to clearing than the control eyes.

In Figure 1 results are expressed as the mean of fibrin clot areas at day 1 for control eyes and annexin V-injected eyes. There was no statistically significant difference between control and 5 μg of annexin V-injected eyes, but fibrin clot areas in the eyes treated with 20 or 60 μg of annexin V (respectively 2·0 and 1·7 quadrants) were lower than the controls in a statistically significant manner ($p<0\cdot01$).

The average days to clearing are shown in Figure 2. Fibrin clearance in control and 5, 20, and 60 μg of annexin V-injected eyes were respectively 4·7, 3, 2·3, and 2·8 days. Differences between control and treated eyes were statistically significant at each dose ($p<0\cdot05$ and $p<0\cdot01$).

COMPARISON OF ANNEXIN V VERSUS HEPARIN

The results of the comparison of the effectiveness of annexin V versus heparin treatment are summarised in Table 1. No statistically significant difference was found between the two treatments.

Discussion

This study shows the effectiveness of annexin V in inhibiting fibrinous postoperative reaction. Intracameral injections of 20 or 60 μg of annexin V resulted in a less important fibrinous reaction than in the control eyes, and this reaction cleared in a shorter time. Injections of 5 μg of annexin V had the same results but the difference between treated and control eyes was not significant for the starting postoperative fibrinous reaction.

Exuberant fibrinous intraocular exudates are a severe complication in vitrectomy surgery or anterior segment surgery and many studies have already investigated the ability of different treatments to prevent or to treat fibrin formation. This was achieved mainly with standard heparin[1,2] or low molecular weight heparin fractions,[7] or with tissue plasminogen activator in the case of treatment.[8,9] In our study we chose to inject 15 IU of standard heparin in the anterior chamber, since it had been shown to be the most effective dose in this protocol.[1] We were unable to show any difference between the annexin V and heparin treated eyes.

Annexin V was already known to have in vitro[5,10,11] and in vivo[12] anticoagulant properties but had never been used in such a model of fibrin formation. The method by which annexin V reduces fibrin formation remains unclear. This effect might be explained by its ability to bind to phospholipids in a Ca^{2+}-dependent manner, a main biochemical feature of all annexins. Phospholipids would then be unable to play their role in the activation of factor X and prothrombin. Thus conversion of fibrinogen into fibrin would then be impaired.

Preoperative and postoperative bleedings are

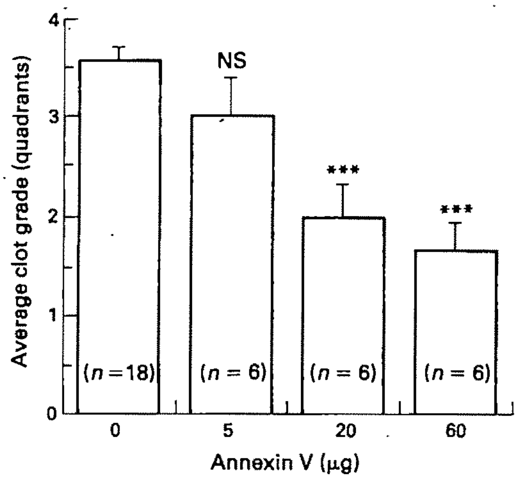

*Figure 1 Fibrin clot areas on the first postoperative day. Results are expressed as the mean (SE) of the fibrin clot areas in control. NS=not significant; ***p<0·01.*

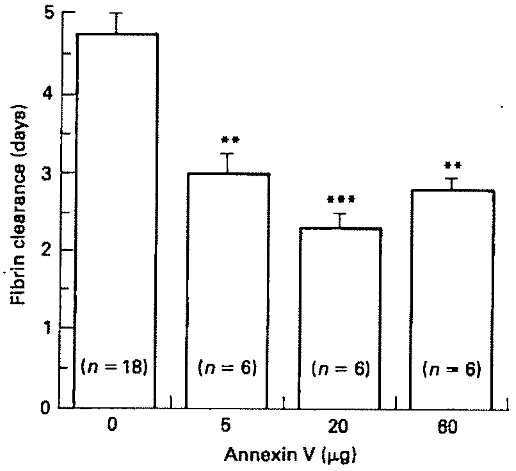

*Figure 2 Time to clearing of the fibrin clot. Results are expressed as the mean (SE) of the number of days to clearing in control eyes. **p<0·05; ***p<0·01.*

Table 1 Efficacy of annexin V versus heparin

Treatment	Average clot grade Mean (SE)	Average days to clearing Mean (SE)
Heparin (15 IU)	2·7 (0·4)	2·8 (0·3)
Annexin V (20 μg)	2·5 (0·5)	2·6 (0·2)

the main reason why standard heparin is difficult to use as a treatment. This explains the great interest aroused by low molecular weight heparin derivatives: they are supposed to lower the occurrence of bleeding while preventing fibrin formation with the same efficacy as standard heparin. In a recent study Iverson *et al*[7] have shown the efficacy of low molecular weight heparin derivatives in the prevention of intraocular fibrin formation occurring after lensectomy, vitrectomy, and retinotomy in a rabbit model. They have effectively noted no increased tendency to postoperatively bleeding. As regards annexin V Römisch *et al*[12] have recently confirmed its antithrombotic effect in an in vivo model of thrombus formation in rats. When investigating bleeding times, they did not find any significant enhancement. For our part, we did not find any tendency to postoperative bleeding in the annexin V-treated eyes. These results remain unexplained and must be confirmed by other studies. However, they would be a very important argument for choosing annexin V as a prophylaxis for intraocular fibrin in settings with high risks of bleeding, as has previously been suggested for low molecular weight heparin.

Furthermore, annexin V has an anti-inflammatory activity through inhibition of phospholipase A_2, a key enzyme in the production of inflammatory lipid mediators. Annexin V seems to operate by protecting phospholipids, which are phospholipase A_2 substrates, from the enzyme activity. Therefore, annexin V may play a role in our study, not only by preventing fibrin clot formation, as shown by the average fibrin clot at day 1, but also by inhibiting the inflammatory processes involved in the postoperative reaction. The shorter time to clearing might be explained in part by this effect. This hypothesis is consistent with the recent study of Chan *et al.*[13] Working on a model of endotoxin-induced uveitis, the authors found that antiflammins, which are synthetic peptides derived from the sequence of annexin I, were efficient in the treatment of phospholipase A_2-mediated intraocular inflammation.

Therefore in this study we have shown the potency of annexin V in reducing fibrin formation in a standardised postoperative model in rabbits. Its efficacy is similar to that of heparin. Annexin V might be considered as a new therapeutic agent of great interest, since it does not seem to enhance bleeding and acts both on fibrin formation and inflammatory reactions.

This suggests further studies to evaluate annexin V in humans and to investigate its efficacy in other ocular pathological settings, where both inflammatory reactions and fibrin formation may occur. Annexin V might also be used coated on intraocular lenses, as is the case for heparin. This is of particular interest since fibrin formation is relatively frequent after extracapsular extraction. Spitznas *et al*[14] recently reported that a pronounced fibrinous reaction occurs in 1·9% of eyes after extracapsular extraction and implantation of a posterior chamber intraocular lens, and that 34·4% of these patients develop posterior synechiae. Lenses coated with an anti-inflammatory and antithrombotic substance might lower the occurrence of this postoperative outcome.

The authors thank A Minty for his helpful comments, A Labat for technical assistance, and M T Guilhem for editing the manuscript.

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Continued breakdown of the blood aqueous barrier following cataract surgery

V M G Ferguson, D J Spalton

Abstract

Following routine extracapsular cataract and posterior chamber implant surgery, recovery of the blood aqueous barrier (BAB) was quantified by sequential anterior chamber fluorophotometry. This was correlated with surgical details and postoperative findings to ascertain those factors which were related to excessive damage of the BAB immediately after surgery and to failure to recover a normal BAB by 3 months postoperatively. A cohort of 84 patients was followed. In the early postoperative period excessive levels of damage to the BAB were related to iris damage ($p<0\cdot01$) and diabetes mellitus ($p<0\cdot01$). By 3 months, 79% of the eyes had recovered normal BABs and 21% (18 eyes) had persisting excessive fluorescence which correlated with an abnormal pupil shape ($p<0\cdot02$) and the development of posterior synechiae ($p<0\cdot001$).

(*Br J Ophthalmol* 1992; 76: 453–456)

Extracapsular cataract surgery with insertion of posterior chamber intraocular lenses causes breakdown of the blood aqueous barrier (BAB) which usually recovers within 3 months of uncomplicated surgery[1-3] Failure to recover the BAB may be associated with a need for continued medication and the potential development of problems such as posterior capsule thickening, cystoid macular oedema, or continuing endothelial cell loss leading to future visual problems. Anterior chamber fluorophotometry measures the degree of BAB breakdown by quantifying the amount of fluorescence in the anterior chamber (AC) following intravenous injection of sodium fluorescein.

In this series 130 patients undergoing routine extracapsular surgery with posterior chamber implants were studied by sequential anterior chamber fluorophotometry during the first 3 months following surgery. The fluorophotometry results were correlated with the clinical findings. The aim was to find out which operative and clinical features were related to excessive damage to the BAB immediately after surgery or failure to recover the BAB by 3 months postoperatively.

Medical Eye Unit, St Thomas' Hospital, London
V M G Ferguson
D J Spalton

Correspondence to:
Mr D J Spalton FRCP, FRCS, Medical Eye Unit, St Thomas' Hospital, London SE1 7EH.

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23 December 1991

Method

Ethical approval was obtained from the West Lambeth Health Authority for the study which took place between December 1987 and June 1989. The patients had no severe cardiovascular or respiratory disease or known allergy and were willing to cooperate with intravenous fluorophotometry, but were otherwise unselected. Extracapsular cataract surgery with posterior chamber implants was performed on 130 patients (130 eyes). The surgical details recorded were the use of preoperative topical indomethacin, the experience of the surgeon, the type of section (limbal or corneal), the fixation of the implant (either in the ciliary sulcus or the capsular bag) and whether a subconjunctival injection of steroid was given.

Patients were studied within 7 days of surgery and at 1, 2, and 3 months postoperatively. At each visit the clinical findings were recorded and AC fluorophotometry was performed. Intravenous fluorescein sodium (20% w/v, 14 mg/kg) was given and fundus fluorescein angiography was performed; 60 minutes later fluorophotometry scans of the anterior chamber were recorded using a 'Fluorotron Master' (Coherent Radiation) fluorophotometer as previously described[1] so that four AC fluorescence readings made at 4 week intervals were produced for each eye.

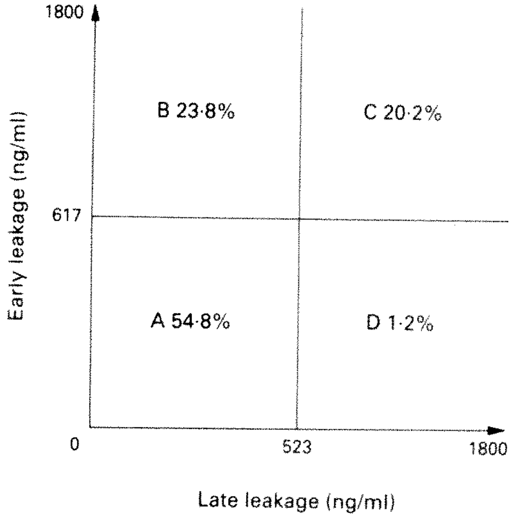
The anterior segment was examined by biomicroscopy and the degree of conjunctival injection, anterior chamber cells, and flare were graded on a scale of 0 to 3. The shape of the pupil, whether round, irregular, or eccentric was recorded. Transillumination of the iris was quantified by retroillumination through the pupil; the iris was divided into eight sectors and the loss of iris pigment epithelium in any sector was recorded immediately after surgery and again at 3 months. The number of sectors with loss of pigment epithelium immediately postoperatively was taken as being surgically induced and progression of these or an increase in affected sectors at 3 months was taken to indicate iris chafing by the implant. Similarly the development of posterior synechiae (PS) at 3 months was graded according to the number of sectors of the iris involved with PS. The centricity of the implant, whether central or eccentric, was noted at 3 months. The best corrected visual acuity at each visit and any opacity of the posterior capsule were also recorded.

Results

A total of 130 patients started and 84 completed the study; 25 patients failed to attend for all four measurements and another 13 declined to complete the study because of nausea caused by intravenous fluorescein. Eight patients were excluded; five because of preoperative uveitis, two highly myopic patients with no implant and one with a dislocated implant which had to be repositioned. There were 31 males, 53 females, (68 Caucasian, nine Negro, seven Asian), aged 43 to 83 years (mean age 67·7 years, SD 9·0).

The normal AC fluorescence to be expected in the eyes of patients of this age was established in

Figure 1 Anterior chamber fluorescence in ng/ml immediately after surgery (y axis) plotted against anterior chamber (AC) fluorescence at 3 months postoperatively (x axis) in a total of 84 eyes. A, B, C and D are the four groups where A and D had relatively low amounts of AC fluorescence in the first week after surgery and B and C had excessive AC fluorescence. At 3 months groups A and B (78·6% of all the eyes) had recovered normal BABs whereas groups C and D (21% of all the eyes) had abnormally high AC fluorescence representing a continued breakdown of the blood aqueous barrier.



a separate study by measuring the AC fluorescence in both eyes of nine healthy volunteers (mean age 62) on two separate occasions. The normal level was 338 plus 186 ng/ml (mean with 2 SD).

Four groups of eyes were identified postoperatively (Fig 1): group A had low AC fluorescence immediately postoperatively and a normal BAB at 3 months (46 out of 84 eyes, 54·8%), group B had high AC fluorescence immediately postoperatively but recovered a normal BAB at 3 months (20 eyes, 23·8%), group C had high AC fluorescence immediately postoperatively and failed to recover a normal BAB at 3 months (17 eyes, 20·2%) and group D started with low AC fluorescence immediately postoperatively but had an abnormal BAB at 3 months (1 eye, 1·2%). Figure 2 shows the range of AC fluorescence values measured within 7 days of surgery. Most eyes (37 out of 84) had levels of fluorescence comparable to unoperated eyes, that is within 338 ng/ml plus 2 SD. Immediately after surgery all the eyes were expected to have some degree of damage to the BAB, but 23·8% had excessively high levels of leakage. An arbitrary level of 617 ng/ml was taken as the threshold for abnormally high fluorescence in the early postoperative period. This represents the mean AC fluorescence for normal unoperated eyes (338 ng/ml plus 3 SD). At 3 months after surgery AC fluorescence of less than 523 ng/ml (mean +2 SD) represented recovery of a normal BAB.

Immediately postoperatively 56% of all eyes (47 out of 84) were in the 'low leakage' group with AC fluorescence less than 617 ng/ml (groups A+D), and 44% had fluorescence greater than 617 ng/ml and formed the 'high leakage' group (groups B+C). At 3 months postoperatively 78·6% of all eyes had recovered a normal BAB (groups A+B) but 21·4% had continued breakdown of the BAB with fluorescence greater than 523 ng/ml (groups C+D); 54% of those with very high fluorescence immediately after surgery subsequently recovered a normal BAB by 3 months (20 eyes out of 37 in groups B+C).

The surgical and clinical findings were compared with the AC fluorescence; firstly in the immediate postoperative period and secondly at 3 months so that the factors related to excessive damage to the BAB immediately after surgery (Table 1) and to continued breakdown of the BAB (Table 2) could be elucidated. The statistical significance of the differences between the groups of eyes was shown using the χ^2 test.

In the early postoperative period the significant factors for high leakage was iris damage ($p<0{\cdot}01$) and the presence of diabetes ($p<0{\cdot}01$). This raised the possibility that the diabetic patients may be the ones with excessive iris damage so a further analysis was made comparing the high leakage and low leakage eyes again but excluding the 10 diabetic patients in the study. The findings were similar with a statistically significant correlation between high leakage and iris damage ($p<0{\cdot}05$). At 3 months the factors related to continued breakdown of the BAB were an abnormal pupil shape ($p<0{\cdot}02$) and the presence of posterior synechiae ($p<0{\cdot}001$) (Table 2). The posterior synechiae were all located in the mid or peripheral iris rather than at the pupil margin.

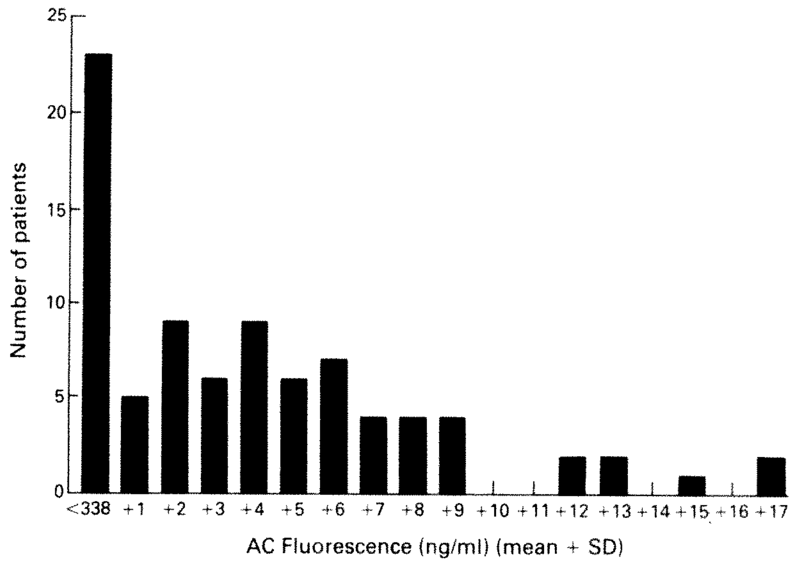
Discussion

Anterior segment fluorophotometry demonstrates the degree of damage to the BAB after routine cataract surgery. Although fluorophotometry has been used extensively to study the effect of cataract surgery on the eye we are not aware of any other study where patients have been studied postoperatively as a sequential cohort. Our results were analysed in two groups: those with high levels of AC fluorescence immediately postoperatively and those failing to recover their BABs by 3 months. In this study we have shown which clinical and surgical factors are related to breakdown of the BAB in the early and late postoperative period.

HIGH FLUORESCENCE IMMEDIATELY POSTOPERATIVELY (TABLE 1)

The presence of diabetes mellitus was related to excessive damage to the BAB immediately after surgery ($p<0{\cdot}001$). Diabetic eyes appear to have more permeable iris vessels than normal eyes and are likely to be more susceptible to surgical trauma. Endothelial cells and basement mem-

Figure 2 Histogram showing the range of levels of anterior chamber fluorescence within the first week after surgery. Each increment represents the mean fluorescence for unoperated eyes plus multiples of the standard deviation.



brane are altered in the iris vessels of diabetic eyes[4,5] and diabetic eyes with retinopathy have more protein in the aqueous and this increases with the severity of the retinopathy.[6] From the clinical point of view Cunliffe *et al*[7] found that diabetic patients had a higher incidence of inflammation and macular oedema post-implantation in comparison to age matched normal eyes.

The second factor correlating with excessive damage to the BAB was trauma to the iris ($p<0{\cdot}01$), shown by changes in the pupillary appearance due to pupillary sphincter damage, iris tucking (caused by the implant, residual capsule fragment or capture of iris in the section) or iris trauma from surgical handling. It is not surprising that iris trauma is so important when one considers that this is a highly vascular tissue which is easily damaged if handled during the operation. By excluding the 10 diabetic patients and repeating the comparison between high and low leakage groups, the significant correlation between iris damage and breakdown of the BAB barrier independent of diabetes was confirmed ($p<0{\cdot}05$).

Table 1 Comparison of patients with normal (Group A+D) and excessive levels of damage to the BAB (Group C+B) immediately after surgery.

Clinical features	*% of patients with feature* *Group A+D*	 *Group B+C*	*p*
Topical preoperative indomethacin	21·3%	18·9	>0·2
Experienced surgeon	63·8	78·4	>0·1
Junior surgeon	36·2	21·6	>0·1
Limbal section	27·7	35·1	>0·2
Corneal section	72·3	64·9	>0·2
IOL fixation			
sulcus	53·2	59·5	>0·2
bag	46·8	40·5	>0·2
Subconjunctival steroid at operation	14·9	21·6	>0·2
Injected conjunctiva in first post-op week	40·4	51·4	>0·2
AC flare (2+) in first week	19·1	8·1	>0·1
AC cells (2+) in first week	21·3	35·1	>0·1
Topical steroid continued >6/52 postoperatively	37·0%	40·5%	>0·2
Postoperative pupil			
round	93·6	73·0	<0·01*
oval	6·4	27·0	<0·01*
Postoperative iris transillumination	8·5	13·5	>0·2
Hypertension	19·1	29·7	>0·2
Diabetes	0	24·3	<0·001*
Development of posterior synechiae (>2+) at 3 months	6·4	21·6	<0·04*
Visual acuity worse than 6/12 at 3 months	4·4%	5·7%	>0·2
Normal BAB at 3 months	97·8%	54%	<0·0001*

Percentages apply to the number of patients in each group to which that particular feature applied. (*=statistically significant with χ^2 analysis).

Table 2 Comparison of patients with normal recovery (groups A+B) to those with continued breakdown of the BAB at 3 months (Groups C+D)

Clinical features	*% of patients with feature* *Group A+B*	 *Group C+D*	*p*
Topical preoperative indomethacin	21·2%	16·7	>0·2
Experienced surgeon	70·2	66·7	>0·2
Junior surgeon	28·8	33·3	>0·2
Limbal section	31·8	27·8	>0·2
Corneal section	66·2	72·2	>0·2
IOL fixation			
sulcus	56·1	55·6	>0·2
bag	43·9	44·4	>0·2
Subconjunctival steroid at operation	13·6	33·3	>0·06
Injected conjunctiva in first postoperative week	45·5	33·3	>0·2
AC flare (2+) in first week	16·7	5·6	>0·2
AC cells (2+) in first week	24·2	38·9	>0·2
IOL position at 3 months			
central	69·7	77·8	>0·2
eccentric	30·3	22·2	>0·2
Pupil appearance at 3 months			
round	89·4	66·7	<0·02*
oval	10·6	38·9	<0·02*
Increased iris transillumination >2+	12·1	27·8	>0·1
Presence of posterior synechiae (>2+) at 3 months	6·1	38·9	<0·001*
Significant posterior capsule opacity at 3 months	4·5	0	>0·2
Topical steroid continued >6/52 postoperatively	39·4	38·9	>0·2
Visual acuity worse than 6/12 at 3 months (excl DM, SMD)	4·5	5·6	>0·2
Diabetes	9·1	16·7	>0·2
Hypertension	21·2	33·3	>0·2
Angiographic CMO at 3 months excluding diabetic or age related maculopathy (grade 2+ or more)	6·3	0	<0·2

Percentages apply to the number of patients in each group to which that particular feature applied. (*=statistically significant with χ^2 analysis.)

Other surgical factors such as the experience of the surgeon, a vascularised limbal or a non-vascularised corneal section, the fixation of the implant or defects in the iris pigment epithelium did not appear to influence the degree of damage to the BAB immediately postoperatively. Also there was no evidence that preoperative treatment with topical indomethacin ($p=0{\cdot}6$) or perioperative injection of subconjunctival steroids ($p=0{\cdot}4$) influenced the degree of damage to the BAB in this early postoperative period. Surprisingly there do not appear to be any studies as far as we are aware on the advantages or otherwise of subconjunctival steroids given at the time of surgery. The effect of indomethacin on the BAB has been extensively studied in terms of its effect on prostaglandins in the aqueous,[8] its effect on the incidence of cystoid macular oedema,[9-11] its effect on the BAB[12] and the benefit of maintaining mydriasis during surgery.[13,14] Indomethacin prevents surgically induced miosis which makes surgery technically easier with less potential for excessive manipulation of the iris and it may be that the majority of the beneficial effects of prostaglandin inhibitors are mediated through this mechanism, particularly as the effects are of relatively short duration. Using pre- and postoperative topical indomethacin Miyake[15] showed reduced AC fluorophotometry measurements in the first 4 weeks postoperatively and Sanders *et al*[2] demonstrated recovery of the BAB by 5 weeks postoperatively in treated eyes compared to untreated eyes which did not show complete recovery of the BAB until 3 months after uncomplicated surgery.

Of all the eyes with high levels of leakage immediately after surgery (group B+C) 46% of them failed to recover their BAB by 3 months. Some of these would probably recover fully with a longer follow up. This initial high leakage group (group B+C) were not given more topical steroids than the low leakage group (group A+D), $p=0{\cdot}8$. They had an increased tendency to formation of posterior synechiae at 3 months ($p<0{\cdot}05$) but their final visual acuities were not different from the low leakage group, $p=0{\cdot}8$.

HIGH AC FLUORESCENCE AT 3 MONTHS (TABLE 2)

Our findings show that at 3 months after surgery 78·6% of all eyes in the study had recovered a normal BAB. Sanders and Kraff[2] also showed that the BAB took 3 months to recover following uncomplicated extracapsular and implant surgery, although only 60 (25%) of their patients completed their 3 month study. We found that persisting breakdown of the BAB at 3 months was associated with an abnormal pupil shape ($p<0{\cdot}02$) and the development of posterior synechiae ($p<0{\cdot}001$). These two factors may be related because posterior synechiae may cause an abnormal shape. The development of posterior synechiae in the high leakage group (C+D) must indicate continuing inflammation as a result of mechanical, immunological, or possibly bacteriological factors. Overall 26% of the eyes developed posterior synechiae in one or more sectors and it is interesting that this percentage compares closely to a post mortem study[16 17] where the authors found histological evidence of inflammatory changes in 31% of eyes with posterior chamber implants. We think that this suggests that the in vivo results from fluorophotometry showing failure to re-establish the BAB by 3 months are reflected by clinical and pathological evidence of continuing inflammatory processes in the eye.

Factors such as the type of section or the position of the implant in the ciliary sulcus or capsular bag were not significantly associated with breakdown of the BAB at 3 months. This is supported by other studies[13 15 18 19] which show that in the early recovery of the BAB after cataract surgery the presence or absence of an implant or its fixation in the bag or sulcus has no significant effect on the BAB. This would indicate that the major factors influencing the degree of damage to the BAB in the early postoperative period are related mainly to surgical techniques. Fixation of the implant is important in long term studies. Miyake[3] looked at fixation of the implant at least 12 months after surgery and found that eyes within the bag placement of the implant had less damage to the BAB than eyes with the implant in the ciliary sulcus, although, with longer follow-up to 3 years, there was no difference between eyes with sulcus or bag fixated posterior chamber implants or even any difference between pseudophakic and aphakic eyes.

One might have expected those eyes in our study who developed an increase in iris pigment loss following surgery (15 eyes) to show increased damage to the BAB. This did not happen and the reason for this is probably that the iris pigment epithelium is avascular and may initially cushion the iris stroma from chaffing. We would anticipate that if chaffing continued those eyes would eventually show damage to the BAB. There was no evidence in this study of an association of posterior capsule opacity with changes in the BAB in the first 3 months after operation which was to be expected as significant posterior capsule opacity is likely to take several months to develop and would occur after the study had been completed. There was an increased incidence of angiographic cystoid macular oedema (CMO) in the eyes with high AC fluorescence at 3 months ($p<0\cdot05$) but when the four eyes with senile macular degeneration or diabetic maculopathy were excluded there was no significant difference between the groups. Our results show an overall incidence of persisting angiographic CMO in 5% (four out of 80) of patients at 3 months. This compares with 6·8% of eyes with 2+ or more angiographic CMO in the study by Wright *et al.*[20]

The results of this sequential study show that breakdown of the BAB immediately after surgery is closely related to the amount of iris trauma during surgery. The degree of iris trauma was independent of diabetes which was also a factor related to increased anterior chamber fluorescence. This emphasises the need for meticulous surgical technique particularly with diabetic patients. At 3 months postoperatively the major factors also appear to be associated with structural changes in the iris and the development of posterior synechiae, probably reflecting continuing inflammatory processes in the eye. Cataract surgery is at present undergoing a major period of change from planned extracapsular surgery to capsulorrhexis and small incision phacoemulsification surgery. A major advantage of this latter style of surgery is potential avoidance of any uveal contact either during surgery or postoperatively and it will be interesting to see if this is reflected by lower degrees of damage to the BAB postoperatively.

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Immunoimaging of choroidal melanoma: assessment of its diagnostic accuracy and limitations in 101 cases

J Scheidler, G Leinsinger, C-M Kirsch, O F Scheiffarth, F H Stefani, K G Riedel

Ludwig-Maximilians-University, Munich, Germany

Department of Radiology
J Scheidler
G Leinsinger
C-M Kirsch

Department of Ophthalmology
O F Scheiffarth
F H Stefani
K G Riedel

Correspondence to:
Dr J Scheidler, Department of Radiology, Klinikum Grosshadern, University of Munich, Marchioninistrasse 15, D-8000 München 70, Germany.

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Abstract

Immunoscintigraphy (IS) was performed on 101 patients with space occupying intraocular lesions including choroidal melanomas (85), choroidal naevi (11), non-melanoma metastases (three), and other melanoma simulating lesions (two). Scintigraphic images with conventional and emission computer tomography techniques were obtained after the intravenous injection of ^{99m}Tc-labelled F(ab')$_2$ fragments of monoclonal antibody (MoAb) 225·28S directed against the high molecular weight-melanoma associated antigen (HMW-MAA). Immunohistochemistry was performed on sections of four out of 10 melanoma-containing eyes to confirm MoAb binding. IS demonstrated positive scans in 66 out of 85 choroidal melanomas, offering a sensitivity of 78%. Sensitivity was dependent on the lesion size. True negative results were obtained in 15 out of 16 non-melanoma lesions (specificity 94%). False positive antibody accumulation was found in one patient with a post-traumatic subretinal haemorrhage. Immunohistochemistry demonstrated positive MoAb 225·28S binding in all melanoma sections. In summary IS offered substantial sensitivity and specificity in the differentiation of intraocular lesions, particularly choroidal melanomas, naevi, and metastases. In combination with other diagnostic procedures such as ultrasound echography and fluorescein angiography IS proved to be a valuable method in the diagnosis of choroidal melanoma.

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At present a variety of diagnostic modalities such as ophthalmoscopy, ultrasound echography, transillumination, and fluorescein angiography are used in the differentiation of intraocular lesions. The combination of ophthalmoscopy, fluorescein angiography, and ultrasound echography provides the highest accuracy.[1] However difficulties still exist in distinguishing a small melanoma from a naevus, a haemangioma, and a metastasis. Echographic characteristics of malignancies are hard to establish when tumour thickness is low or not determinable.

Radioisotopes in the diagnosis of ocular melanoma were formerly extensively used in the ^{32}P test. Both, the necessity of a surgical procedure to place a probe onto the sclera for β-count measurement and some false positive results, due to the non-specific tracer accumulation, raised doubts of its clinical value.[2] Another attempt was the administration of ^{51}Cr bleomycin, a non-specific oncophilic radiopharmaceutical. Sensitivity and specificity were reported to be satisfactory.[3] Patients in an advanced stage of the disease however were also included and no data on the lesion size were provided.

The invention of monoclonal antibodies (MoAbs) by Köhler and Milstein[4] and the formation of MoAbs against tumour-associated antigens added a new dimension to specific in vitro and in vivo tumour diagnosis. For in vivo application MoAbs can be labelled with γ-emitting isotopes and used as tracers for immunoscintigraphic tumour detection. Recent publications demonstrated the clinical value of the new diagnostic method in numerous malignant and benign diseases – for example, colorectal carcinoma, ovarian carcinoma, cutaneous melanoma, infection, thrombosis, and human cardiac transplant rejection.[5-10]

Up to now there are no specific MoAbs available against choroidal melanoma. However immunohistological studies of Bomanji[11] and Scheiffarth[12] showed the binding of MoAb 225·28S raised against cutaneous melanoma in choroidal melanoma. This class IgG$_2\alpha$ antibody recognises the high molecular weight-melanoma associated antigen (HMW-MAA), a membrane-bound glycoprotein which is present on most cutaneous melanomas and to a lower extent in skin naevi and skin carcinomas.[13] First preliminary in vivo studies by Bomanji[14] and Scheidhauer[15] were promising, but also emphasised the importance of further studies to evaluate the sensitivity and in particular the specificity of immunoscintigraphy.

This study was performed to define the value of immunoscintigraphy (IS) using the MoAb 225·28S for the diagnosis of choroidal melanoma in comparison with established diagnostic modalities and to determine the minimum lesion size for reliable imaging.

Patients and methods

A total of 101 patients with suspected choroidal melanoma were examined by IS after written consent had been obtained. Patients with a history of allergic reactions were excluded. Prior to the intravenous administration of the radiolabelled antibodies, the thyroid gland was blocked with perchlorate (50 drops). In most cases diagnosis was verified by ophthalmoscopy, ultrasound echography, and fluorescein angiography. Clinical follow-up in all non-melanoma lesions lasted more than 1 year. Ultrasound echography was performed according to the method described by Ossoinig[1] using the A- and B-image. Ten patients underwent enucleation because of the large tumour size.

The MoAb 225·28S (F(ab')$_2$ fragments), directed against the HMW-MAA was delivered as a ready-to-use kit (Technemab K1; Sorin

Biomedica, Italy). Approximately 350 μg of lyophilised antibodies were reconstituted by sterile insertion of 2 ml sodium ^{99m}Tc-pertechnetate solution into the vial. Activity ranged between 700–1250 MBq according to the time of imaging. After 15 minutes of incubation at room temperature, free ^{99m}Tc-pertechnetate was separated with a DEAE-Sephadex A25 ion exchange chromatography column. Labelling yield was 70–80%. Then 450–1000 MBq of radiolabelled antibody were diluted with isotonic NaCl solution to a total volume of 5 ml and slowly injected intravenously.

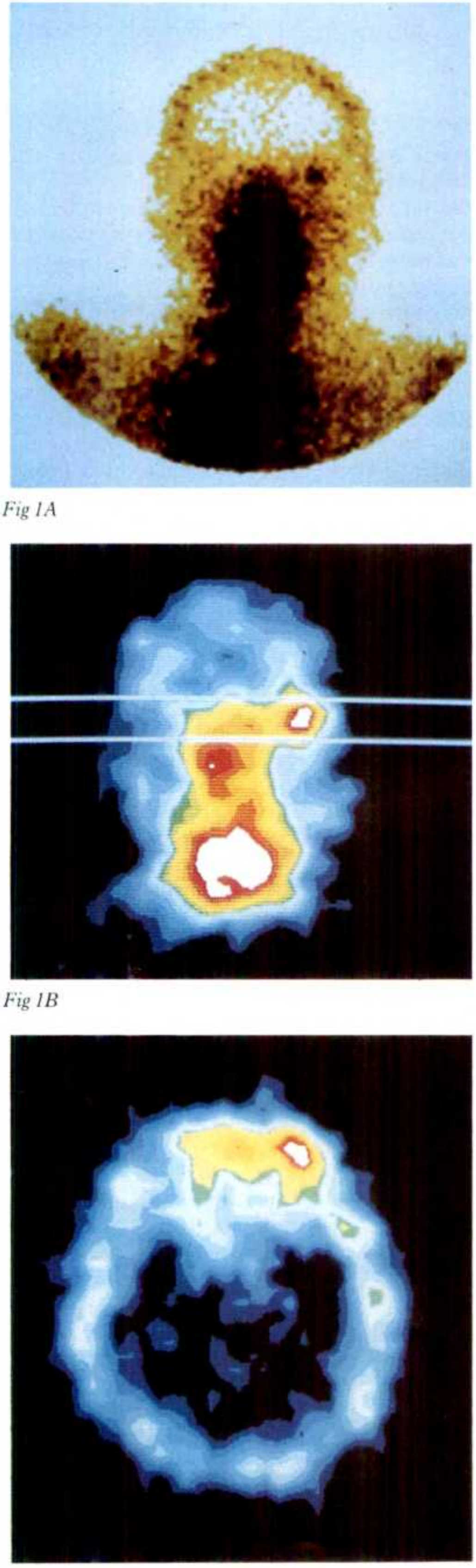

Fig 1A

Fig 1B

Fig 1C

Figure 1 A 69-year-old female patient. Antibody accumulation in a choroidal melanoma of the left eye, thickness 5·7 mm. Conventional imaging (A) and SPECT images, frontal (B) and transverse sections (C).

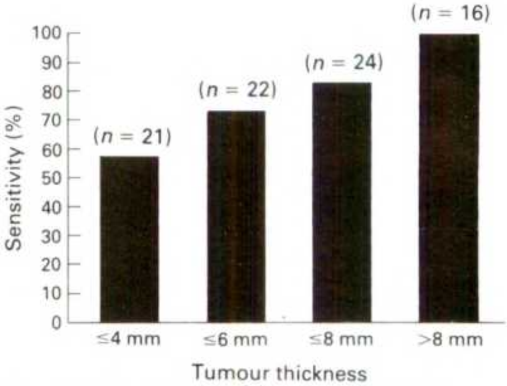

Figure 2 Correlation between thickness of choroidal melanomas, measured by ultrasonography and sensitivity of immunoimaging.

For imaging the patient was placed in a supine position with the head fixed in a foam support with a caudal tilt of 15–30°. This position was chosen to avoid attenuation effects of the cranium and interference with non-specific activity uptake in the nasopharynx. Imaging was carried out 6 or 16 hours after antibody administration for practical reasons and from previous experience in cutaneous melanoma.[16,17] Static images were obtained with a large field of view gamma camera equipped with a high resolution collimator designed for low energies. Acquisition time was 15 minutes. A 128×128 computer matrix was utilised for data processing. Additionally, in all patients single photon emission computer tomography (SPECT) was performed. Either a single head (Gamma-tome, CGR) or a double-head (Rota, Siemens) rotating gamma camera system equipped with a high resolution collimator (parallel hole, 140 keV) was used. For single head acquisition images were acquired by continuous rotation over 360° in 40 minutes and for the double head system by rotation in step and shoot mode (360°, 60 angular projections, 25 second acquisition/projection) around the patient. A total of at least 300 000 counts for study in a 64×64 matrix were collected. Transverse sections were reconstructed by a filtered back-projection algorithm (Sheep-Logan-Hanning filter, cut-off frequency at 0·5, no attenuation correction) with subsequent computation of coronal and sagittal slices. Images were interpreted prospectively in a blind fashion without knowledge of the results of ophthalmoscopy and ultrasound echography and with no clinical information other than an instruction to rule out melanoma.

Cryostat sections of four out of 10 enucleated melanomas were investigated immunohistochemically to demonstrate MoAb binding. After incubation with anti-melanoma MoAb 225·28S (dilution 1:20) and second rabbit anti-mouse antibody sections were stained in alkaline-anti-alkaline phosphatase (APAAP).[18]

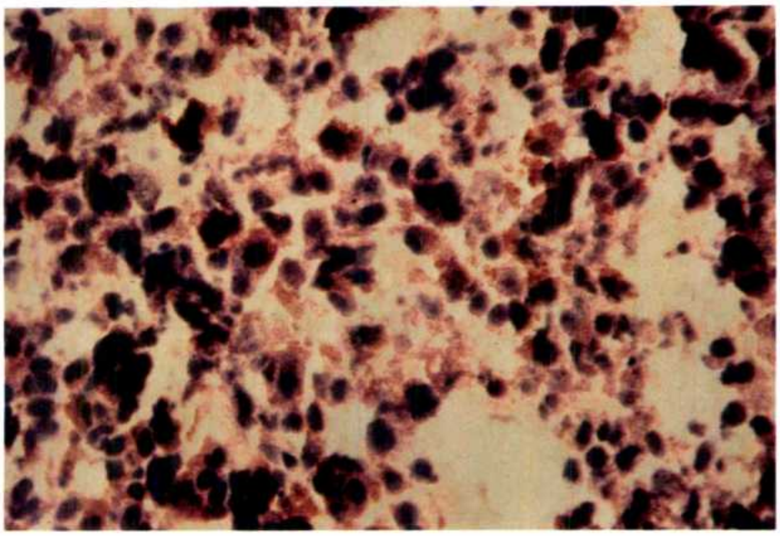

Figure 3 Immunohistochemistry with MoAb 225·28S on choroidal melanoma section (APAAP reaction, dilution 1:20) counterstained with Mayer's haematoxylin. Magenta cytoplasmal staining indicates MoAb binding to HMW-MAA. Counterstaining is responsible for black colour reactions of nuclei.

Results

Space occupying intraocular lesions investigated by IS included choroidal melanomas (85), naevi (11), non-melanoma metastases (three), and post-traumatic subretinal haemorrhages (two). Maximum lesion thickness measured by ultrasound echography ranged from 0 mm (not detectable) to 23 mm (mean 5·4 mm). No clinical signs of metastases were observed in all the melanoma patients. In 10 patients the melanoma-containing eye was enucleated with the clinical diagnosis confirmed by histopathology. All other patients were treated by irradiation with a ruthenium–106 scleral applicator.

No side effects or adverse reactions were noticed after the application of the antibody. In this study no data could be collected on the induction of human anti-mouse antibodies (HAMA) because there was no repeated antibody administration. However none of our patients with cutaneous melanoma who underwent repeated administration of the same MoAb under similar conditions had HAMA.

Sixty six out of 85 melanomas were visualised by the SPECT imaging technique (sensitivity 78%). However only 35 of them were also detected by planar imaging. This results in a gain of the SPECT technique of 47%. As expected in this stage of disease no brain metastases were diagnosed by IS. Non-specific tracer uptake was visualised in the nasopharynx, liver, kidneys, and bone marrow (Fig 1).

Detectability has shown a positive correlation with the thickness of the lesions measured by ultrasound echography: ≤4 mm 57%, ≤6 mm 73%, ≤8 mm 83%, and >8 mm 100% of choroidal melanomas were positive in IS. Melanomas with a thickness below 2·7 mm (n=2) were not detected. Imaging was performed 6 or 16 hours following antibody application. Analysis of results revealed a higher sensitivity for early compared with delayed imaging, but this fact can be attributed to a different tumour size (7·6 mm vs 6·1 mm). No significant difference in sensitivity was seen between melanomas of nasal or temporal location: 86% of nasal (mean thickness 6·9 mm) and 81% of temporal located melanomas (mean thickness 5·7 mm) were visualised (Fig 2).

Table 1 Results of immunohistochemistry (IH) with the MoAb 225·28S (APAAP method) compared to immunoscintigraphy (IS) with the same radiolabelled MoAb. Immunohistochemistry was possible on four out of 10 enucleated melanomas only

Patient	*Lesion size (mm)*	*IS*		*IH*
		planar	*SPECT*	
M.R.	8.9	+	+	+
J.R.	12·5	+	+	+
B.A.	8·0	–	–	+
L.I.	10·8	+	+	+

Fifteen of 16 non-melanoma lesions were truly negative (specificity 94%). A slight accumulation of radioactivity in both eyes was seen in one patient with bilateral naevi in the SPECT slices only. This accumulation was not considered to be a positive result. All other naevi and the non-melanoma metastases were negative. One false positive result was obtained in an old subretinal post-traumatic haemorrhage simulating a tumour mass (thickness 7·4 mm). IS could demonstrate the HMW-MAA in all sections examined (Fig 3 and Table 1).

Discussion

Our data show that lesions of a size even below an order of magnitude of resolution can be visualised. Thus the rate of antibody uptake seems to be the decisive parameter for tumour detection by IS. According to a recent study[12] the expression of HMW-MAA was reported in all melanoma sections examined by immunohistochemistry though choroidal melanoma exhibited antigenic heterogeneity.[19] Considering the small number of melanomas tested in all immunohistological studies, this suggested heterogeneity can be responsible for a sensitivity of IS below 100%.

Conventional planar scintigraphy proved not to be sufficient for immunoscintigraphic imaging of choroidal melanomas. Taking into account the small size of melanomas, SPECT, the most advanced imaging technique in nuclear medicine, was used additionally and compared with conventional scintigraphy. SPECT images are created by a technique similar to x ray computer tomography, which offers the selection of defined sections in transversal, frontal, and sagittal projection. Compared with planar scintigraphy SPECT provides equal at-best resolution, improved contrast without superimposed background activity, and better three-dimensional orientation. However, even with the SPECT imaging technique intraocular melanomas smaller than 2·7 mm in size were invisible.

Although brain metastases can be demonstrated by IS, non-specific activity accumulation makes the exclusion of liver metastases impossible. Occurrence of non-specific tracer uptake in liver, nasopharynx, and bone marrow is due to the well-known transchelation of DTPA-bound ^{99m}Tc to transferrin and its accumulation in the reticuloendothelial system. Activity accumulation in the kidneys representing the physiological route for the excretion of antibody fragments ($F(ab')_2$). Surprisingly, there was no significant difference in sensitivity between melanomas of nasal or temporal location as the non-specific nasopharyngeal uptake did not interfere with the detection of nasally located lesions.

All tumour markers used at present are not specific for a certain tumour, they are just expressed in a higher quantity by malignancies compared with normal tissue. The HMW-MAA is also present in skin naevi. Up to now there are no immunohistological data regarding the presence of this antigen in choroidal naevi. Normal eyes do not react with antibodies against the HMW-MAA.[12] In our study only one out of eight naevi revealed a slight antibody uptake while all intraocular metastases were negative. These results are in agreement with Bomanji,[20] who reported antibody accumulation in a large flat choroidal naevus. The presence of this antigen may indicate malignant transformation of the naevus. In the 2 year follow-up of this patient no clinical signs for tumour progression were encountered. Intensive activity accumulation was visualised in a second non-melanoma lesion, a subretinal haemorrhage following trauma. Haemorrhages can possess a higher blood pool which may be a possible explanation for this false positive result.

In conclusion, IS with MoAbs against the HMW-MAA proved to be a valuable diagnostic method which offers substantial sensitivity and specificity for the differentiation of intraocular space occupying lesions, particularly choroidal melanoma, naevus, and metastasis. It may add valuable information when the diagnosis cannot be confirmed by other diagnostic methods. Prospects for the future are the development of MoAbs against tumour antigens specific for choroidal melanoma[21] as well as the injection of antibody cocktails against different epitopes,[22] or the application of melanoma-specific haptens[23] to improve immunoimaging of choroidal melanoma.

In memory of Dr O F Scheiffarth.

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Detection of colour vision abnormalities in uncomplicated type 1 diabetic patients with angiographically normal retinas

K J Hardy, J Lipton, M O Scase, D H Foster, J H B Scarpello

North Staffordshire Royal Infirmary, Department of Endocrinology and Diabetes Mellitus
K J Hardy
J H B Scarpello

Department of Ophthalmology
J Lipton

University of Keele, Staffordshire, Department of Communication and Neuroscience
M O Scase
D H Foster

Correspondence to:
Dr K J Hardy, Department of Medicine, Division of Endocrinology and Metabolism, University of New Mexico, Albuquerque, New Mexico 87131, USA.

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Abstract

Colour vision function was assessed in 38 non-complicated type 1 diabetic patients in whom fluorescein angiography was normal, and was compared with that in 36 age-matched, non-diabetic controls. All of the patients were healthy and none were taking medication except insulin. The eye examination, which was normal in every patient, included the Ishihara and City University tests, measurement of Snellen acuity, slit-lamp examination, tonometry, and fundal photography as well as fluorescein angiography. Colour discrimination ability was measured with the Farnsworth-Munsell 100-hue test. Mean (SE) 100-hue test error score for the diabetic group was 86·8 (8·1) compared with 28·2 (3·3) for controls, $p \ll 0·001$. There was no relation between colour vision abnormalities and diabetes duration ($r=0$, $p>0·05$), blood glucose at the time the colour tests were performed ($r=0·4$, $p>0·05$), most recent glycated haemoglobin result ($r=0·3$, $p>0·05$), or the mean of all previous glycated haemoglobin results ($r=0$, $p>0·05$). It is concluded that colour discrimination may be abnormal in uncomplicated type 1 diabetic patients before the onset of retinopathy, and that colour discrimination losses in diabetes may not be of vascular aetiology.

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Most reports concerning the aetiology of diabetic retinopathy have been concerned with its vascular aspects or their sequelae.[1] Little evidence exists for a mechanism independent of vascular changes as a cause of diabetic retinal malfunction. For several years it has been known that colour discrimination is abnormal in diabetic patients with either background retinopathy,[2] or with proliferative retinopathy and maculopathy.[3] In both groups of patients there is overt vascular damage (diabetic retinopathy) which may be the cause of the colour discrimination losses.

The aim of the present study was to determine whether colour vision is abnormal in young, and non-complicated type 1 diabetic patients without any evidence of vascular disease, and without retinopathy as defined by fluorescein angiography.

Patients and methods

PATIENTS

We compared 38 type 1 diabetic patients aged between 16 and 40 years with 36 age-matched, non-diabetic controls. Table 1 summarises the clinical details of diabetic patients. Controls were recruited from staff and students at the local university, and staff at the local hospital. There were 23 males and 13 females. All were aged between 16 and 40 years (mean age 25·3 (SD 4·3) years), and all were healthy. None of the controls were taking any form of medication (including the oral contraceptive pill). All the diabetic patients were healthy, and none were taking any medication (including the oral contraceptive pill) except insulin. No patient had evidence of any complications of diabetes, in particular none were hypertensive, or had nephropathy or microalbuminuria; the urinary microalbumin: creatinine ratio (Laurell-Rockett Electrophoresis, laboratory reference range <2·1) was used as a measure of urinary microalbumin excretion and was normal in all patients. Glycated haemoglobin (Corning method, laboratory reference range 5·5–8·2) was used to assess blood glucose control. The protocol was approved by the district ethical committee, and all subjects gave written informed consent.

EYE EXAMINATION

Eye examination comprised measurement of corrected Snellen acuity, tonometry, slit-lamp examination of the lens and anterior chamber, fundal photography, and fluorescein angiography. Control subjects were assessed by measures of corrected Snellen acuity and by direct ophthalmoscopy.

COLOUR VISION TESTS

We tested colour vision on a separate occasion to avoid temporary changes in visual function which may result from slit-lamp examination[4] and fluorescein angiography.[5] We used traditional clinical tests: the Ishihara 38-Plate Test (Kanehara & Co Ltd, Tokyo, Japan), the City University Test, 2nd Edition (Keeler, Windsor, UK),

Table 1 Clinical details of the 38 diabetic patients in whom colour discrimination was studied

	Diabetics
Number	38
Sex	27 male (11 female)
Age (years)	26·1 (1·1)[*]
Age of diabetes onset (years)	18·3 (1·3)
Diabetes duration (years)	8·1 (0·8)
Systolic blood pressure (mm Hg)	123 (2)
Diastolic blood pressure (mm Hg)	75 (1)
Insulin dose (units/kg)	0·72 (0·04)
Serum cholesterol (mmol/l)	4·9 (0·1)
Urinary microalbumin to creatinine ratio	1·1 (0·3)
Glycated haemoglobin (%)	10·2 (0·3)

[*] Mean (SE).

Table 2 Colour vision test results in diabetics without retinopathy and in non-diabetic controls

	Diabetics (n=38)	*Controls (n=36)*	*Significance*
Ishihara test	38/38	38/38	NS
City University test	10/10	10/10	NS
100-hue test error score (untransformed)	86·8 (8·1)*	28·2 (3·3)*	p≪0·001
Square-root transformation of 100-hue test error score	8·98 (0·3)	4·87 (0·3)	p≪0·001
Age-corrected 100-hue test error score	39·9 (8·6)	−12·4 (3·7)	p≪0·001

*Mean (SE).

and the Farnsworth-Munsell 100-hue test (Kollmorgen Corporation, Baltimore, MD, USA). Each patient performed the tests with one eye, selected at random by computer, under standardised lighting conditions (North Skylight, illuminance 1680 lx, CIE coordinates $x=0{\cdot}3198$, $y=0{\cdot}3282$, correlated colour temperature=6127 K (Northlight, Thorn EMI Lighting, London, UK)). The Farnsworth-Munsell 100-hue test was administered according to the original instructions.[6] A polar plot of each subject's score was examined visually and was submitted to Fourier analysis to examine for evidence of polarity.

STATISTICAL ANALYSIS

Statistical analysis was by Student's t test (two-tailed) for normally distributed data. The Mann-Whitney U test was used for data that were not normally distributed. Raw error scores for the 100-hue test are usually positively skewed[7] and a square-root transformation is often performed to normalise the data. As the appropriateness of this square-root transformation has recently been questioned[8] both raw error scores and transformed data are presented here.

Results

By virtue of the conditions of entry into the study, all the diabetic patients had normal Snellen acuity (better than 6/9), an intraocular pressure of less than 21 mm Hg, and normal ocular media. Fluorescein angiography was also normal in all patients (a single microaneurysm being considered abnormal). Control subjects had normal Snellen acuity (better than 6/9) and no abnormalities of the ocular media or of the retina on direct ophthalmoscopy.

All subjects scored normally on the Ishihara 38-plate test and the City University Test. Results for the 100-hue test in the form of total error scores and their transformations are shown in Table 2. In addition, an age-adjusted mean error score based on Verriest's original norms[9] is included. This adjusted score must be regarded as an approximation only since our illumination was higher than that used by Verriest. Most (57%) of the diabetic group had 100-hue error scores more than 2 SD values above the mean for the control group.

A polar plot of the 100-hue error score was obtained for each subject, and typical plots for three diabetic patients are shown in Figure 1. No individual patient had evidence of polarity on his or her 100-hue plot, nor did Fourier analysis of individual data reveal any significant polarity (2-cycle/0-cycle <0·2 in all cases; see Allen[10]).

By virtue of the conditions of entry in the study, the age-range of the patients and their age-matched controls was limited [mean (SD) 26 (7) years]. There was no correlation between age and diabetes duration ($r=0{\cdot}09$, $p>0{\cdot}05$), nor between age and 100-hue error score ($r=0{\cdot}12$, $p>0{\cdot}05$). As shown in Figure 2, there were no significant correlations between 100-hue error score and diabetes duration, blood glucose at the time of colour vision testing, most recent glycated haemoglobin, or the mean of all previous glycated haemoglobin results.

Discussion

Marked abnormalities of colour vision were detected in young, uncomplicated type 1 diabetic patients in whom fundal photography and fluorescein angiography were normal. The relatively low error scores in the control group compared with the normative data of Verriest[9] may be due in part to the rigorous exclusion criteria employed in this study. No control (or patient) had evidence of lens abnormalities, which may occur earlier in diabetic patients[11] and may influence colour discrimination.[12] The age range was limited to subjects between 16 and 40 years, for although age has an influence on colour discrimination[9] it varies little over this range. Many drugs,[13] including the oral contraceptive pill,[14] and oral hypoglycaemic agents,[13] may

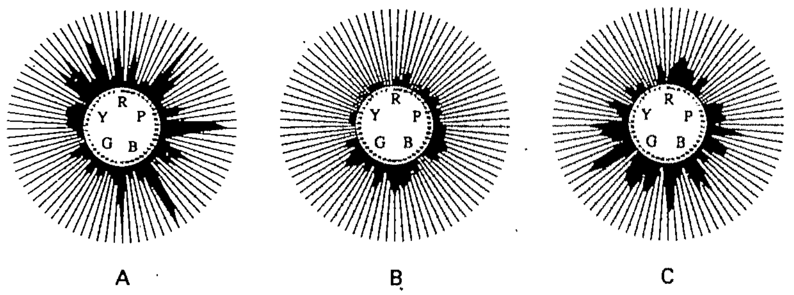

Figure 1 Typical polar plots of errors in the 100-hue test for three diabetic patients. Different regions of the colour circle are indicated by letters (R=red, P=purple, B=blue, Y=yellow, G=green) with the corresponding cap numbers indicated (2, 4, 6, etc). Note that there is no systematic pattern to the distribution of the errors.

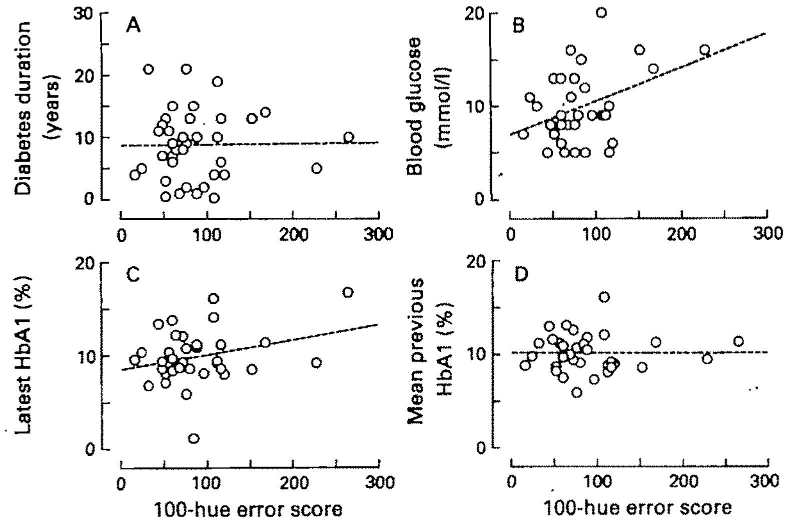

Figure 2 Graphs showing the relationship between colour discrimination ability (100-hue error score) and (A) diabetes duration, (B) blood glucose at the time of colour vision assessment, (C) latest glycated haemoglobin (HBA1) result, and (D) the mean of all previous HBA1 results, for 38 diabetic patients without retinopathy.

adversely affect colour vision, so that subjects taking medication other than insulin were excluded. Our use of a relatively high level of illumination may also have contributed to the relatively low error scores for the controls. Colour discrimination with the 100-hue test is known to vary with level of illumination[15]: low levels produce higher error scores, and may introduce an artefactual tritan-like colour discrimination loss[16]; a higher level was used here to avoid these effects, and also because it was more appropriate for the other colour vision tests we employed. Verriest acknowledged that he had used a lower level of illumination than was usual in his 1963 study,[17] and that the illuminant in his 1982 study was to compromise between his 1963 illuminant and levels of illumination used by others. Indeed, in a study using the City University colour vision test (also employed in this study), he recommended an illumination level close to that used here.[18] There were slightly more males in the diabetic group than in the control group, but colour discrimination does not differ significantly between men and women.[17] Thus, the present findings cannot be attributed to unusually good performance in the control group. Even when performance in the 100-hue test was compared with Verriest's original 1963 data for normal controls, the error scores in the patient group were significantly worse.

Previous studies have shown that diabetic patients with retinopathy, and thus with overt vascular disease, may have abnormal colour discrimination.[2,3] In this study, none of the young type 1 diabetic patients had any evidence of either macrovascular or microvascular disease, yet colour discrimination was markedly abnormal in 57% of patients. It is possible that despite a lack of any evidence of vascular disease a few patients may have had occult vasculopathy. It is unlikely, however, in view of the low age of the group and their short duration of diabetes that occult vascular disease could have accounted for the impaired colour discrimination present in so many patients. It is known that with increasing diabetes duration the microvascular complications of diabetes become more frequent.[19–21] No association between colour discrimination loss and duration of diabetes was present in this study, nor have previous studies found such an association.[2,22] The incidence of microvascular complications is increased in those patients with the highest glycated haemoglobin values,[23] particularly when an aggregate of previous glycated haemoglobin results is used.[24] Like earlier studies of colour vision in diabetes,[2,25] there was no relationship, in this study, between either the latest glycated haemoglobin result, or the mean of all the previous glycated haemoglobin results, and colour discrimination ability. The evidence from this study thus supports the view that colour discrimination losses in diabetic patients without retinopathy may be of a non-vascular aetiology. By what mechanism could these colour vision changes be mediated? Several possibilities exist. (1) There may be osmotic distortion of the retina. Impairment of colour discrimination ability might result from fluid shifts in the retina causing distortion and thus dysfunction of the neural cells, in the same way that acute changes in blood glucose lead to refractive errors secondary to osmotic distortion of the lens.[26] This hypothesis would be supported by changes in colour discrimination ability occurring at the time of acute changes in blood glucose. This change of colour vision with blood glucose level has so far been convincingly demonstrated only during hypoglycaemia,[27] when the effects may have resulted directly from neuroglycopenia. (2) There may be disordered metabolism of neural cells. Although the cause of neuronal dysfunction in diabetic neuropathy is unclear, diabetes may damage the nerve directly, or indirectly through changes in the microcirculation. Interest has recently focused on the sorbitol pathway, and on aldose-reductase inhibitors which inhibit the conversion of glucose to sorbitol. The use of aldose-reductase inhibitors in the management of diabetic neuropathy in humans has so far been only modestly encouraging,[28,29] and in a randomised, placebo-controlled study of 41 diabetics, treatment with ponalrestat for 1 year did not improve visual dysfunction.[30] Another recent therapy in the treatment of diabetic neuropathy has been dietary supplementation with the essential fatty acid derivative, γ linoleic acid (GLA). This and other essential fatty acid derivatives are important constituents of the phospholipids of neuronal membranes (and of the cone-receptors); and it is known that their formation is impaired in diabetes. Studies in animals have shown that diabetic neuropathy can be prevented and in some cases reversed by the administration of GLA,[31,32] and in humans GLA is significantly better than placebo in the treatment of diabetic neuropathy.[33] It is possible that abnormalities of essential fatty acid metabolism are also involved in the development of diabetic retinopathy, and it is interesting to note that almost 30 years ago King *et al*[34] demonstrated a significant reduction in exudative retinopathy in patients treated with fatty acid supplements.

In summary, this study has demonstrated marked abnormalities of colour vision in diabetic patients in whom there was no evidence of retinopathy or vascular disease. This suggests that the aetiology of early colour vision loss is not vascular. Whether retinal dysfunction is directly attributable to changes in blood glucose, or whether it results from derangement of other metabolic pathways is as yet unknown.

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Abnormal dark adaptation kinetics in autosomal dominant sector retinitis pigmentosa due to rod opsin mutation

Anthony T Moore, Fred W Fitzke, Colin M Kemp, Geoffrey B Arden, T Jeffrey Keen, Christopher F Inglehearn, Shomi S Bhattacharya, Alan C Bird

Abstract

The time course of dark adaptation was measured in 10 subjects from three families with autosomal dominant sector retinitis pigmentosa (RP) due to mutations in the first exon of the rod opsin gene. In each subject cone adaptation and the early part of the recovery of rod sensitivity followed the normal time course, but the later phase of rod adaptation was markedly prolonged. The recovery of rod sensitivity is much slower than that reported in any other outer retinal dystrophy. Using a model based upon primate data of rod outer segment length and turnover, we have calculated that the delayed phase of the recovery of rod sensitivity in the RP patients tested following strong light adaptation could be due in part to formation of new disc membrane with its normal concentration of rhodopsin rather than in situ regeneration of photopigment.

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Research into disease mechanisms in retinitis pigmentosa (RP) is complicated by heterogeneity within the disorder. RP may be inherited as an autosomal dominant, autosomal recessive, or X-linked disorder, and there is good evidence of heterogeneity within genetic subtypes.[1-13] Further subdivision of autosomal dominant RP (ADRP) has been achieved by analysis of functional deficits. Two broad categories of RP have been identified which are designated as class I or 'diffuse' and class II or 'regional' forms,[1-4] and the functional characteristics appear to be consistent within families indicating that the functional differences reflect genetic heterogeneity. An additional variant, sector RP is characterised by retinal atrophy seen in only one part of the fundus, usually the lower nasal quadrant, and gross field loss confined to the area of visual field corresponding to the involved retina. The rod and cone electroretinographs show mild reduction in amplitude with normal cone implicit times.[5-7] This pattern of disease is seen in all affected family members, irrespective of age suggesting that the disease, in contrast to other forms of ADRP, is non-progressive or progresses very slowly.

Recent genetic studies have provided evidence for further heterogeneity in AD and X-linked RP.[8-12] About 25% of families with ADRP show mutations of the rod opsin gene on chromosome 3,[13 14] and among this group of patients different patterns of retinal dysfunction may be seen with different mutations.[15-20] Recently it has been shown that genes other than that for rod opsin may transmit ADRP.[21 24]

Here we report the clinical, electroretinographic, and psychophysical findings in 10 patients from three families with autosomal dominant sector RP due to mutations in the rod opsin gene. In each of these families there was markedly slowed rod adaptation.

Patients and methods

Ten affected individuals from three families diagnosed as having sector ADRP were studied (Table 1). Informed consent was obtained after the nature of the procedure had been fully explained. In each patient ophthalmoscopy revealed pigment epithelial atrophy and pigment migration into the lower retina which was most marked in the inferior nasal quadrant. The upper retina had a normal appearance. In each case Goldmann perimetry revealed upper field loss corresponding to the ophthalmoscopically abnormal retina (Fig 1). Electroretinography was performed in nine subjects in accordance with a standard protocol[25] (Table 2). Dark adapted perimetry was performed on the right eye of each subject using red (dominant wavelength 660 nm, subtending 0·9°) and green (dominant wavelength 530 nm, subtending 0·9°) targets. The pupil was dilated with 1% cyclopentolate and the eye dark adapted for 40 minutes before starting the test. At least 17 points at different retinal locations in both upper and lower fields were tested in each case. The apparatus and method for the dark adapted static perimetry have been described previously.[26] Dark adaptation curves were obtained from threshold measurements determined using the green (530 nm, 0·9°) target of the static perimeter or the Tubinger perimeter (dominant wavelength 500 nm; stimulus size 1·7°). We chose to determine the dark adaptation curves at points in the clinically uninvolved retina where the rod threshold elevations were less marked (Table 1). Threshold measurements were made on the right eye. The pupil was dilated, the eye dark adapted for 60 minutes, and a dark adapted threshold was then determined. The area of retina to be tested was then fully light adapted (7·5 log scotopic trolands for 3 seconds) and measurements were continued in the dark for at least 60 minutes (or longer if the patient could tolerate it).

Blood samples were collected from each subject and DNA extracted. Exon sequences were amplified using the polymerase chain reaction (PCR) and screened for mutations using the heteroduplex assay as described pre-

Addenbrookes Hospital, Cambridge
A T Moore
C M Kemp

Institute of Ophthalmology, London
F W Fitzke
G B Arden
A C Bird

Department of Human Genetics, Newcastle
T J Keen
C F Inglehearn
S S Bhattacharya

Correspondence to:
Mr A T Moore, MA FRCS, Ophthalmology Department, Addenbrookes Hospital, Hills Road, Cambridge, UK.

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Table 1 Pre-bleach thresholds and elevation of threshold after 60 minutes of dark adaptation following strong light adaptation. In families 1038 and 1084 the mutation is Gly106Arg, and in 1935 it is Thr58Arg

Subject	*RP family*	*Age*	*Test location*	*DA threshold elevation*	*Additional threshold elevation*•
1	1038	36	180M 25E	0·8	1·0
2	1038	64	330M 25E	1·1	1·2
3	1038	74	315M 20E	1·9*	1·1*
4	1084	26	180M 25E	0·9	1·6
5	1084	47	345M 25E	1·1	1·0
6	1935	45	315M 40E	0·6*	0·8*
			315M 50E	0·5*	0·4*
7	1935	18	180M 25E	0·1	1·9
			180M 40E	0·0	1·4
8	1935	16	180M 40E	1·1	1·6
9	1935	21	315M 30E	0·0	1·3
10	1935	50	315M 40E	1·5*	1·4*
			315M 50E	1·5*	2·1*

•Additional threshold elevation=the elevation over the dark adapted threshold after 60 minutes of adaptation following bleach.
DA=dark adapted; M=meridian; E=eccentricity measured in degrees. Thresholds were measured with a LED adaptometer*, or Tubinger perimeter.

Table 2 Amplitudes and implicit times of the electroretinograms

Subject		*Dark adapted blue*		*Bright flash white*				*Flicker (30 Hz)*	
Eye	*Age*	*b amp*	*b imp*	*a amp*	*a imp*	*b amp*	*b imp*	*b amp*	*b imp*
1 R	36	220	60	160	24	360	50	35	30
L		200	60	120	24	340	50	40	30
3 R	74	200	80	140	26	260	64	10	40
L		180	80	100	27	200	56	15	40
4 R	26	280	60	200	13	420	60	50	30
L		240	60	160	13	400	60	50	30
5 R	47	320	60	160	20	300	50	50	30
6 R	45	110	62	80	22	140	51	20	32
L		115	70	80	22	140	56	20	33
7 R	18	170	74	120	22	280	50	35	30
L		160	74	160	22	280	50	45	30
8 R	16	230	68	140	22	280	60	100	30
L		200	68	160	22	320	60	80	30
9 R	21	170	80	50	22	230	50	25	32
L		100	70	60	22	160	50	35	32
10 R	50	100	84	55	24	180	54	12	34
L		80	84	60	24	140	55	11	36
Normal values									
		319	57	264	15	404	50	43	29
(1 SD)		(89)	(6)	(64)	(2·5)	(88)	(3)	(5)	(1·5)

amp=amplitude in μV; imp=implicit time in milliseconds; no data are available on subject 2.

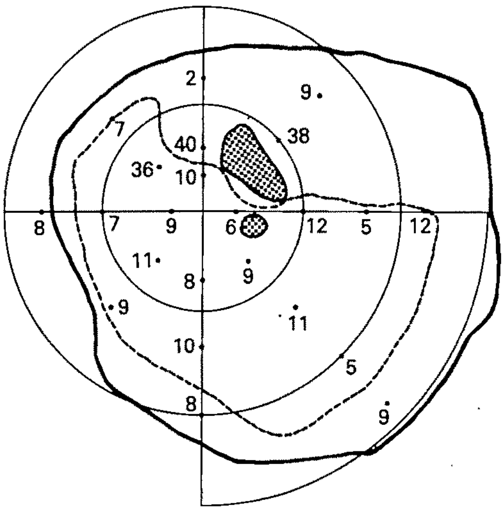

Figure 1 Patient 1: right eye, Goldmann visual fields to IV4e (solid line) and IL4e (broken line) targets. Numerical values indicate dark adapted static perimetry threshold elevation in tenths of a log unit to a green (530 nm) target).

viously.[27,28] Pairs of oligonucleotide primers were synthesised surrounding rod opsin exons to give PCR products in the range of 200–300 bp. The PCR was carried out using a two-stage reaction profile of 94°C for 30 seconds and 60°C for 3 minutes, repeated 30 times. Aliquots of the PCR reactions mixes were then electrophoresed on D-5000 hydrolink gels (AT Biochem, Malvern, PA, USA for around 2000 Vh). Mismatches due to the presence of a heterozygous mutation caused shifted mobility in heteroduplexed molecules, which could then be further characterised by sequencing the appropriate PCR product.

Results

Rod opsin mutations were identified in each family comprising a change of threonine to arginine at codon 58 in RP1935, and glycine to arginine at codon 106 in RP1038 and RP1084. Both mutations can be detected as altered restriction sites in PCR amplified rod opsin exon 1 (Fig 2).

In all subjects Goldmann visual field loss was confined to the upper field, and dark adapted perimetry confirmed that thresholds for both the 530 nm and 660 nm targets were markedly elevated throughout that region (Fig 1). In the lower field, which was normal on Goldmann perimetry, dark adapted perimetry was normal in some areas but mildly abnormal in others consistent with the regional pattern of disease. This pattern of threshold elevation was a consistent finding in all 10 subjects.

All subjects had normal or mildly reduced rod and cone b-wave amplitudes of the ERG (Table 2). There was a trend for the amplitudes to become less with age but they did not approach the levels seen in non-sector RP. Rod b-wave implicit times were abnormal in all five members of RP1935 and the oldest member of RP1038. Cone implicit times were normal in at least one member of each family.

Dark adaptation was markedly prolonged in all 10 subjects, and none reached their pre-bleach threshold by 60 minutes. At this time the threshold elevation relative to the pre-bleach values ranged from 0·4 to 2·1 log units (Table 1). Inspection of the dark adaptation curves showed that in each case there was a similar pattern of abnormality; cone adaptation was normal, the rod cone break occurred at the normal time, and the early part of the rod adaptation curve followed an apparently normal time course. Subsequently rod adaptation was markedly slowed with a gradual drift down towards the threshold (Figs 3 and 4).

Because of the difficulty with prolonged testing it is not possible to give the exact time for full recovery of rod function. However two subjects agreed to be tested again during the following days. At the end of the initial dark adaptation determinations the subjects returned to normal ambient lighting conditions, and were later retested at the same retinal location at periods ranging from 24 to 120 hours post-bleach. On each occasion the subjects were dark adapted for 1 hour before threshold measurements were taken. Subject 10 showed elevated thresholds of 0·6 and 1·4 logs units above pre-bleach values at two different locations 44 hours after light adaptation, and in subject 7 the threshold was elevated 1·5 log units at 24 hours but had returned to the dark adapted value by 120 hours.

Discussion

Nyctalopia is a prominent and early symptom in

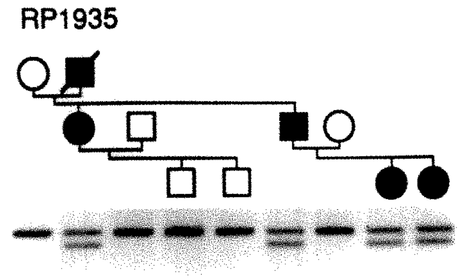

Fig 2A

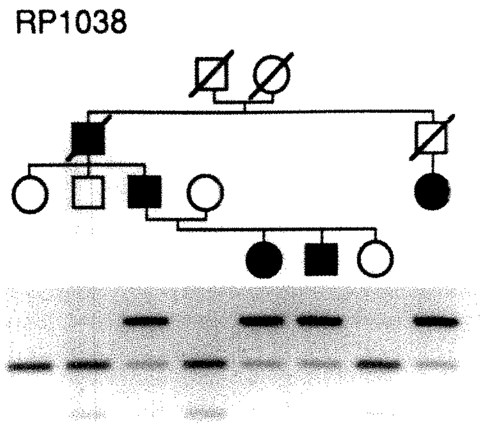

Fig 2B

Figure 2 (A) Family 1935 pedigree, mounted above a photograph of an ethidium bromide stained gel showing PCR amplified exon 1 from members of the family cut with the enzyme DdeI. PCR primers CATGTTTCTG CTGATCGTGC and ACTCTCCCAGACCCC TCCAT give a 295 bp fragment which is normally uncut with the restriction enzyme Dde I. However the codon 58 ACG to AGG mutation introduces a Dde I site, which gives rise to 254 and 41 bp bands. All affected individuals are heterozygous for the uncut and cut fragments. (B) Family 1038 pedigree, mounted above a gel picture showing PCR amplified exon 1 from family members which has been cut with the restriction enzyme ApaI. The same PCR primers were used giving a fragment of 295 bp, which is normally cut to 191 and 104 bp fragments by Apa I. The codon 106 GGG to AGG mutation destroys this site, so that affected individuals are again heterozygous for digested and undigested product.

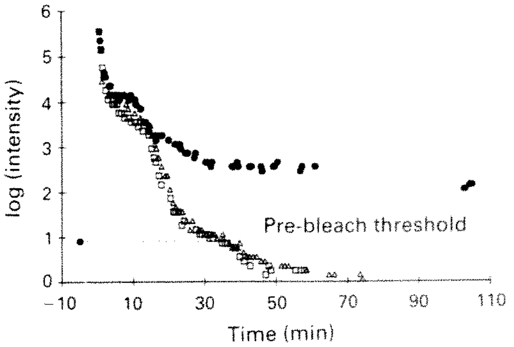

Figure 4 Patient 4: right eye, dark adaptation curve plotted using the Tubinger perimeter (500 nm wavelength) at 25 degree eccentricity along horizontal nasal field meridian. The patient's threshold values (solid circles) and those of two normal observers (open symbols) are plotted relative to normal absolute threshold. The patient's baseline dark adapted threshold is shown as the horizontal line. Adaptation to the 8.0 log scotopic troland seconds light was at time zero.

RP, and elevated absolute thresholds are seen in all types of the disease. Where there is extensive loss of rod function, cones may mediate threshold even under scotopic conditions so that the dark adaptation curve is monophasic. In patients with less advanced disease, most studies have identified a biphasic dark adaptation curve with a normal time course but elevated final threshold.[29 30] However, Alexander and Fishman pointed out that unless a baseline dark adapted threshold is determined before light adaptation it is not possible to be certain that dark adaptation is complete even when thresholds appear to have stabilised.[31] They identified several patients with different types of RP in whom the final thresholds were elevated and the time course of recovery of rod sensitivity was slow. They did not determine that the abnormality was consistently seen within a family or genetic subtype.

Using a similar approach we have been able to show delayed rod dark adaptation in all 10 subjects from three families with autosomal dominant RP. Our patients resemble those designated as sector RP[5-7] in that altitudinal distribution of disease was found consistently in each family whatever the age of the subject. Although the implicit time of the rod and cone b-waves were not normal in all subjects, normal times were found in at least one member of each family.

The functional abnormality in our patients with a mutation at codon 106 is qualitatively similar to that seen with the mutation at codon 58. Slow rod adaptation has been identified previously in some patients with ADRP,[19 20 31 32] including some with altitudinal distribution of disease and known mutations of the rod opsin gene namely: threonine-17-methionine[19]; proline-23-histidine,[20] and threonine-58-arginine.[19] Our results are similar to those previously reported although the slow phase of recovery of rod sensitivity was longer in our patients than in each of the other genotypes. There is no clear explanation for this disparity although the light adaptation protocols were different in that we used more light (8·0 log scotopic troland seconds as opposed to 7·5), and it was delivered in a shorter time (3 seconds as opposed to 60).

There are several possible mechanisms by which abnormality of the rod opsin molecule could result in slowed adaptation. There may be slowed regeneration of rhodopsin as seen in fundus albipunctatus[33 34] or delayed removal of abnormal bleach photoproducts which may interfere with rhodopsin formation or desensitise the rod photoreceptors.[31]

Alternatively, it is possible that abnormal rod opsin could interfere with normal disc assembly, structure, or stability. The prolongation of the recovery of rod sensitivity to a period of several days led us to consider whether or not outer segment renewal may contribute to the later phase of dark adaptation in our patients. In our model we have assumed the dark adapted rod

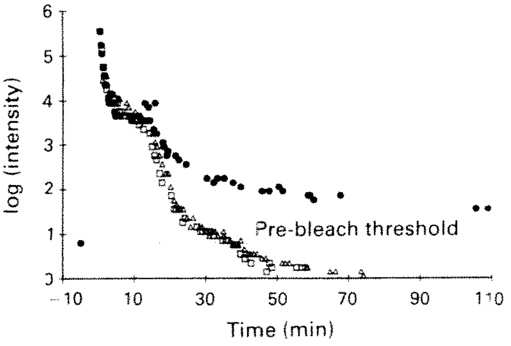

Figure 3 Patient 1: right eye, dark adaptation curve plotted using Tubinger perimeter (500 nm wavelength) at 25 deg eccentricity along the horizontal nasal field meridian. The patient's threshold values (solid circles) and those of two normal observers (open symbols) are plotted relative to normal absolute threshold. The patient's baseline dark adapted threshold is shown as the horizontal line. Adaptation to the 8·0 log scotopic troland seconds light was at time zero.

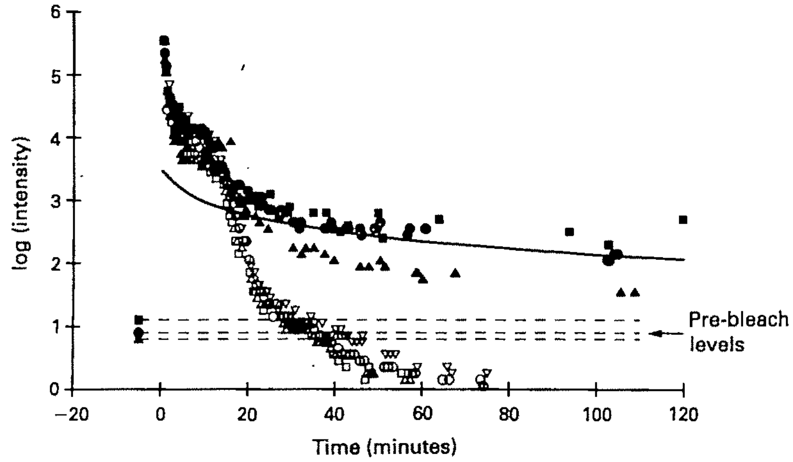

Figure 5 Dark adaptation curves measured under similar conditions to Figures 2 and 3 showing the results from three patients (subjects 1, 4, 8) compared with normal controls (open symbols). These patients are illustrated since dark adaptation was recorded for more than 100 minutes. The pre-bleach thresholds are indicated by the horizontal lines. The solid curve represents the results of the calculation of the effect on threshold of outer segment renewal (see text).

threshold elevation can be completely accounted for by reduced levels of rhodopsin causing decreased absorption of incident quanta proposed in the regional form of disease.[4] This supposition is consistent with the observations of Kemp *et al.*[20] If there were either no significant in situ regeneration of rhodopsin after full light adaptation, or excessive shedding from a shortened outer segment leaving only a greatly truncated functioning outer segment, recovery of rod sensitivity would depend largely on the formation of new outer segment discs with their normal concentration of rhodopsin. In favour of the latter is the suggestion that night blindness in some forms of RP may be caused by progressive shortening of the rod outer segment due to an imbalance between disc shedding and renewal,[35] and the identification of shortened rod outer segments in autosomal dominant RP in a histological study.[36] Using data from primate studies of rod outer segment length and disc turnover,[37] the recovery of rod sensitivity due entirely to regrowth of photoreceptor discs can be calculated. It is possible then to model the effect of different rod outer segment lengths and different rates of renewal on recovery of rod sensitivity. Here we have considered a simple model of an outer segment length of 0·01 μm following intense light adaptation and assumed normal outer segment renewal of 3 μm per day. The predicted recovery of rod sensitivity with formation of new outer segment discs can be calculated using the quantum catch relationship[4] (Fig 5). Using this simple model there is a good fit between the predicted recovery of rod sensitivity and that actually measured in our subjects during the entire abnormal late phase of rod adaptation.

Thus, the very slowed late phase of rod dark adaptation seen in our subjects, may be explained by the regrowth of rod outer segments with their normal concentration of rhodopsin. The data do not distinguish between the two possible mechanisms, namely failure to regenerate rhodopsin on existing outer segment disc membranes and light induced shedding restricting available membrane. However the normal time course of the early phase of recovery of rod sensitivity is more readily explained by the second; if shedding were not complete and in situ regeneration of rhodopsin occurred normally in the small residual outer segment, initial rod dark adaptation would be normal as seen in our patients. The similarity between the recovery of sensitivity predicted by the model and that seen in our patients suggests that outer segment renewal contributes to recovery after light adaptation in some patients. To date this mechanism of recovery from strong light adaptation has received little attention, and is worthy of further study.

The model requires that the outer segments are short as a results of RP and that a major portion of the outer segment is shed following strong light adaptation. The mechanism by which alteration of the amino acid sequence of the rod opsin molecule may influence outer segment structure or stability remains to be elucidated. The phenomenon occurs with different mutations in the rod opsin molecule, although all mutations associated with this pattern of disease recorded to date are on the first exon, and it is quite possible that similar functional defects may be found with defects on other genes.

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Confocal imaging of the fundus using a scanning laser ophthalmoscope

W H Woon, F W Fitzke, A C Bird, J Marshall

Institute of Ophthalmology, Judd Street, London WC1H 9QS
W H Woon
F W Fitzke
A C Bird
J Marshall

Correspondence to:
W H Woon, Department of Ophthalmology, St Thomas' Hospital, London SE1 7EH.

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Abstract

A confocal scanning laser ophthalmoscope (cSLO) was used to examine the effects of confocal optics on the image of the human fundus in vivo. Patients from a retinal clinic and a glaucoma clinic were examined using the cSLO in the confocal mode. A degree of optical sectioning could be achieved, and the results agree with a best axial resolution of 300 μm measured in a model eye. The main advantage of using a confocal system was found to be the improved contrast of the images. This improved the resolution of structures such as the lamina cribrosa and optic disc drusen which are seen in low contrast in conventional images. The improved contrast of the confocal images is partly achieved by excluding light which has been scattered within the plane of focus. Structures which multiply scatter light will become less visible with confocal optics and hard exudates were found to be an example of such a structure. The cSLO and the fundus camera are seen as complementary instruments rather than as alternatives for imaging the fundus. It is envisaged that confocal imaging will enable details of the fundus to be revealed which are at present not seen in conventional images.

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Confocal imaging is an established technique for obtaining high resolution images in the laboratory.[1] In vivo confocal imaging of the human fundus can now be used by the ophthalmologist with the introduction of a commercially available confocal scanning laser ophthalmoscope (cSLO). The scanning laser ophthalmoscope uses a focused laser beam to scan over the area of the fundus to be imaged.[2-4] At any instant only a small spot on the fundus is illuminated, and the light returned from this spot inside the eye determines the brightness of a corresponding point (pixel) on the TV monitor (Fig 1). If the laser is made to scan over successive points on the fundus then an image can be built up as an array of pixels on the TV monitor. This is done by using a spinning mirror and an oscillating mirror, and the laser beam is made to scan in a raster fashion across the fundus. The angle through which the laser beam is scanned determines the area of the fundus that is scanned and therefore the field of view. A large scanning angle will give a large field of view and therefore a low magnification. The Rodenstock 101 cSLO can operate in a 20 degree field of view mode or a 40 degree field of view mode.

If the photodetector in the SLO accepts all the light leaving the eye under examination, then the image is said to be non confocal (Fig 2, top). Non-confocal images suffer from loss of contrast due to light which is returned to the detector after being scattered by media opacities or after multiple scattering in the fundus. To improve

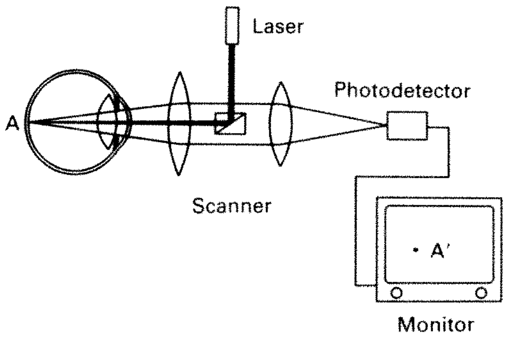

Figure 1 The SLO uses a laser beam to illuminate the fundus. At any instant only a single point (A) is illuminated. Light returned from the eye is collected by a photodetector, and the point A is represented by a pixel A' on the TV monitor.

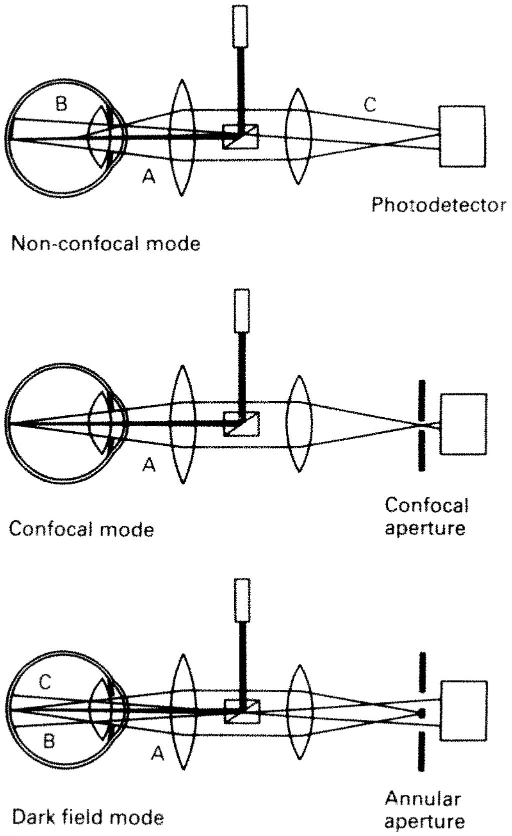

Figure 2 Top: non-confocal mode. The photodetector accepts a high proportion of the light scattered back from the eye. Ray A is light scattered from the laser focus on the retina. Ray B is light which has been side scattered from the laser focus before being rescattered out of the eye. Ray C is light which has been scattered from the optical media. Rays B and C do not directly come from the laser focus and reduce the contrast of the image of the fundus. Middle: confocal mode. A pinhole (confocal aperture) placed conjugate to the laser focus at the fundus ensures that only rays of light (A) directly scattered back from the laser focus pass to the photodetector. Bottom: dark field mode. An annular aperture blocks out rays of light (A) directly scattered back from the laser focus. Rays of light (B, C) which have been side scattered from the laser focus before being rescattered out of the eye can pass through to the photodetector.

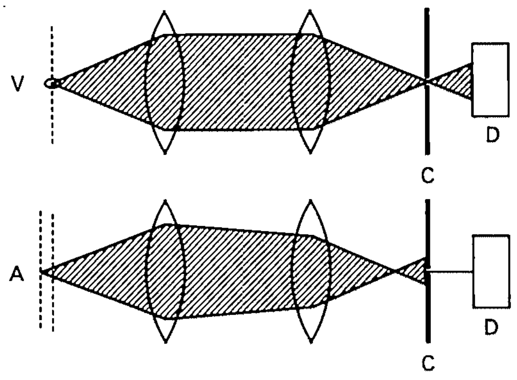

Figure 3 Top: the confocal aperture (C) ensures that only light from a small volume or voxel (V) reaches the detector (D). Bottom: point A is not conjugate to the confocal aperture and most of the light from point A is blocked by the confocal aperture. Light from structures outside the plane of the voxels will not contribute to the image.

image contrast the detector should only receive light that is scattered back directly from the laser focus on the fundus. This is achieved by placing a pinhole, which is conjugate to the laser focus, in front of the detector[3,4] (Fig 2, centre). The pinhole is called a confocal aperture and the image so obtained is said to be confocal. The instantaneous volume of tissue from which back scattered light is accepted by the confocal aperture is called a voxel (Fig 3, top). The size of the confocal aperture determines the degree of confocality of the image, a small confocal aperture gives rise to a small voxel and a highly confocal image.[5] In a confocal system, the detection unit is instantaneously accepting light from a single point on the fundus, and the detection system must be made to scan in synchrony with the laser illumination so that the detection system and illumination system are directed at the same point on the fundus. This is achieved by the double scanning of the cSLO which uses the same scanning system in reverse directions for the illumination and detection systems.[3,4]

In the confocal mode, an image is built up of light scattered back from a layer of voxels, and the image is thus taken from a thin section of equal thickness to the depth of a voxel. In this way an optical section is produced, as light from structures outside the plane of the voxels will not contribute to the image (Fig 3, bottom). This may be contrasted with conventional imaging where objects outside the depth of field contribute a blurred image and can degrade the image of objects within the depth of field. The thickness of the optical section will depend on the confocality of the image. A smaller confocal aperture will give a narrower optical section within the limits imposed by the optics of the human eye.[6,7] The axial position of the optical section may be altered by altering the plane that is conjugate to the confocal aperture. In the Rodenstock 101 cSLO, this may be done by using the ametropic corrector. Adding negative power to the ametropic correction will move the plane of focus deeper in the eye, and vice versa. The ability of confocal imaging to separate structures in different optical sections gives it axial resolution, though imaging an optical section deep to the surface of an object is only possible for transparent or translucent objects.

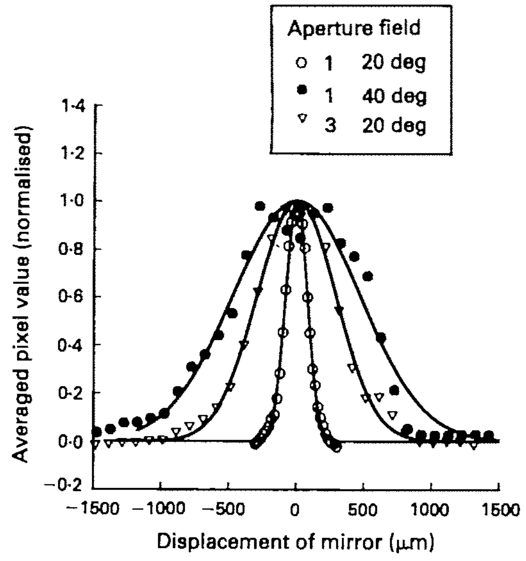

Figure 4 Axial resolution of the cSLO measured in a model eye. The best axial resolution is achieved using confocal aperture 1 in the 20 degree field of view mode.

The cSLO can be used in a mode which produces an image which is effectively the image produced by subtracting the confocal image from the non-confocal image. This mode is called the dark field mode and is produced by using an annular aperture in place of the pinhole confocal aperture.[3] The annular aperture blocks out light directly scattered back from the laser focus while allowing light which has been rescattered from an area adjacent to the laser focus to pass through (Fig 2, bottom). Thus the dark field mode detects multiply scattered light, and objects which have many scattering centres will appear bright in this mode.

The images from the presently available cSLO are monochromic, and differences in spectral information are lost. In addition, a confocal image differs from a conventional monochromatic image because the contrast in a confocal image arises mainly from differences in reflection and refraction rather than differences in absorption of the incident light.[8] This means that the confocal image does not show the same variation with wavelength that is found with monochromatic fundus camera images. In a confocal image blood vessels show up well using red illumination, while this is not true for conventional monochromatic images. This is particularly important as the red HeNe laser is most commonly used in cSLOs.

The visibility of a structure depends on its contrast with its surroundings. Thus, although the resolution of the cSLO may be less than the resolution of a conventional fundus camera for structures which are seen in high contrast, the cSLO may be able to resolve structures which cannot be resolved by conventional imaging because of poor contrast.

We have used a commercially available cSLO to assess the value of confocal imaging of the fundus. The axial resolution was measured in a model eye, and confocal images were obtained from patients.

Methods

The axial resolution of the cSLO was measured using a method that has been previously described.[9] A model eye was used which consisted of a 16 mm lens and a micrometer mounted mirror. The pixel brightness of the cSLO image of the mirror was measured for a range of axial positions of the mirror.

Fifty one patients were examined using a cSLO. The patients were recruited from a retinal clinic and a low tension glaucoma clinic. Patients from the retinal clinic were already dilated with tropicamide 1% and phenylephrine 10%, while patients from the low tension glaucoma clinic were dilated with tropicamide 1% if required. The patients were examined with a Rodenstock 101 cSLO using the HeNe laser alone. Images were recorded using a U-matic video recorder (Sony VO-5850P).

Optical sectioning of the live fundus was performed using the 20 degree field of view and confocal aperture 1. The axial position of the optical section was varied by altering the setting of the ametropic corrector.

A range of pathological conditions was examined using the confocal mode of the cSLO. The ametropic corrector was used to correct for ametropia, and to focus on the structure of interest. Images were produced using a range of confocal apertures and both the 20 degree field and 40 degree field of view mode. Images were compared with colour slides obtained using a fundus camera. In selected patients, enhanced confocal images were also produced by computer averaging over 16 frames. This was done using an IBM AT personal computer with a FG100 frame grabber. The program was supplied by the Eye Research Foundation, Boston. The frames were aligned manually before averaging. No other computer enhancement was performed on the images. Hard copies of averaged images were obtained by photographing (FP4 film) the video monitor (Sony PVM 1440QM).

Results

The cSLO was not difficult to learn to use. The level of retinal irradiance was comfortable, with several patients remarking spontaneously that they preferred the cSLO to the fundus camera. It was found that pupillary dilatation was helpful when using the cSLO in the 20 degree field of view mode, but was not necessary when using the 40 degree field of view.

The axial resolution of the cSLO in the model eye is shown in Figure 4. The axial resolution is measured from the points where the brightness of the image of the mirror has fallen by a half. The best axial resolution (full width at half maximum, FWHM) is 300 μm for the 20 degree field of view and the smallest confocal aperture (aperture 1). In practice, when using confocal aperture 1 and the 20 degree field of view, the image quality suffered from insufficient light levels, even with maximum illumination. Light levels using larger confocal apertures in the 20 degree field mode or confocal aperture 1 in the 40 degree field mode were found to be sufficient.

Optical sectioning was most easily demonstrated in imaging the disc in patients with low tension glaucoma. It was possible to obtain high quality images of the lamina cribrosa in all 10 patients with low tension glaucoma by focusing deep to the retina (Fig 5, top). The lamina cribrosa was visible using conventional ophthalmoscopy, but was difficult to photograph using a fundus camera. In two patients it was possible to image the lamina cribrosa underlying the neuroretinal rim using confocal optics (Fig 5, centre). Improved contrast was also demonstrated in a patient with optic nerve head drusen and a patient with an optic disc melanocytoma (Fig 5, bottom and Fig 6, top).

The improvement in contrast with confocal imaging is best demonstrated in imaging the retinal vessels. The contrast could be improved by reducing the size of the confocal aperture (Fig 7).

Vitreous floaters were often seen using the cSLO. In one patient with low tension glaucoma, a vitreous discontinuity with macular attachment could be demonstrated (Fig 6, bottom).

In three patients with hard exudates, it was found that the exudates became less visible using confocal imaging, and that the exudates were well seen using the dark field mode (Fig 6, centre, and Fig 7).

It was found that, subjectively, a single frame image captured from the video tape was not of comparable quality to the dynamic video recording. Computer averaging over 16 frames produced images that were subjectively of comparable quality to the dynamic video recording. No new details were seen in the averaged images.

Discussion

The cSLO provides a novel method of imaging the fundus. At present, when used purely for non-dynamic imaging, the cSLO should be considered as complementing rather than replac-

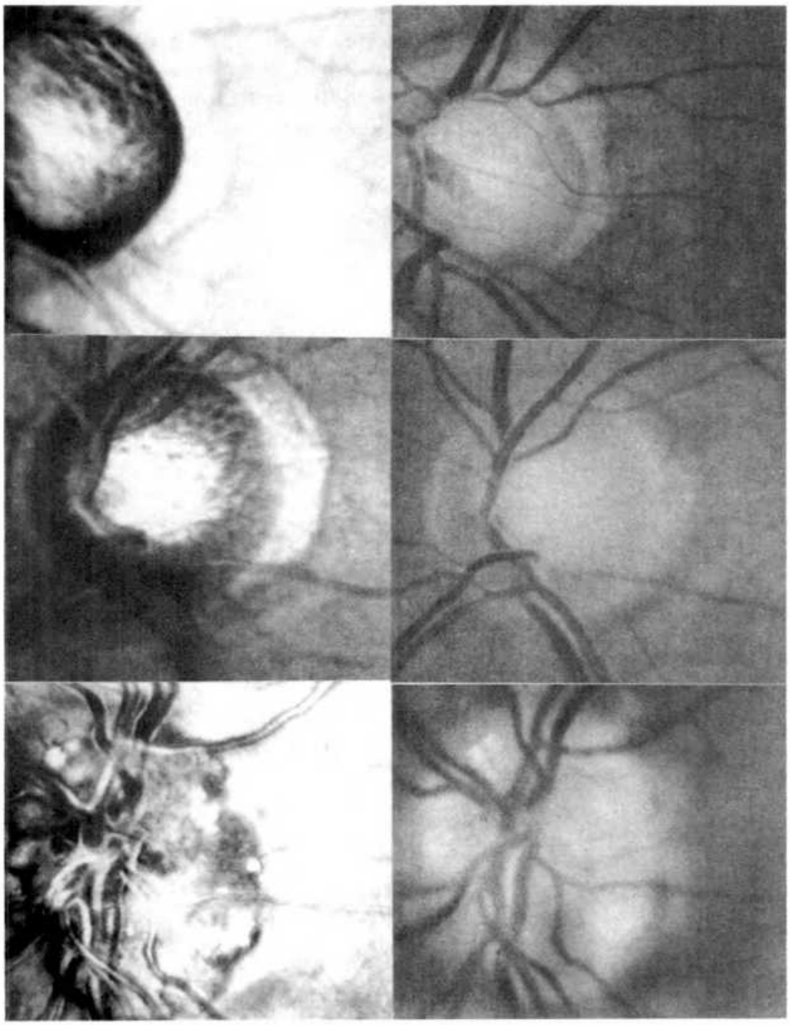

Figure 5 Top: confocal (left) and fundus camera (right) image of the disc in a subject with low tension glaucoma. The lamina cribrosa is seen in higher contrast in the confocal cSLO image. Centre: confocal (left) and fundus camera (right) image of a normal disc. The lamina cribrosa is visible underlying the neuroretinal rim in the confocal cSLO image. Bottom: confocal (left) and fundus camera (right) image of disc drusen. The nodular structure of the drusen is apparent in the confocal cSLO image.

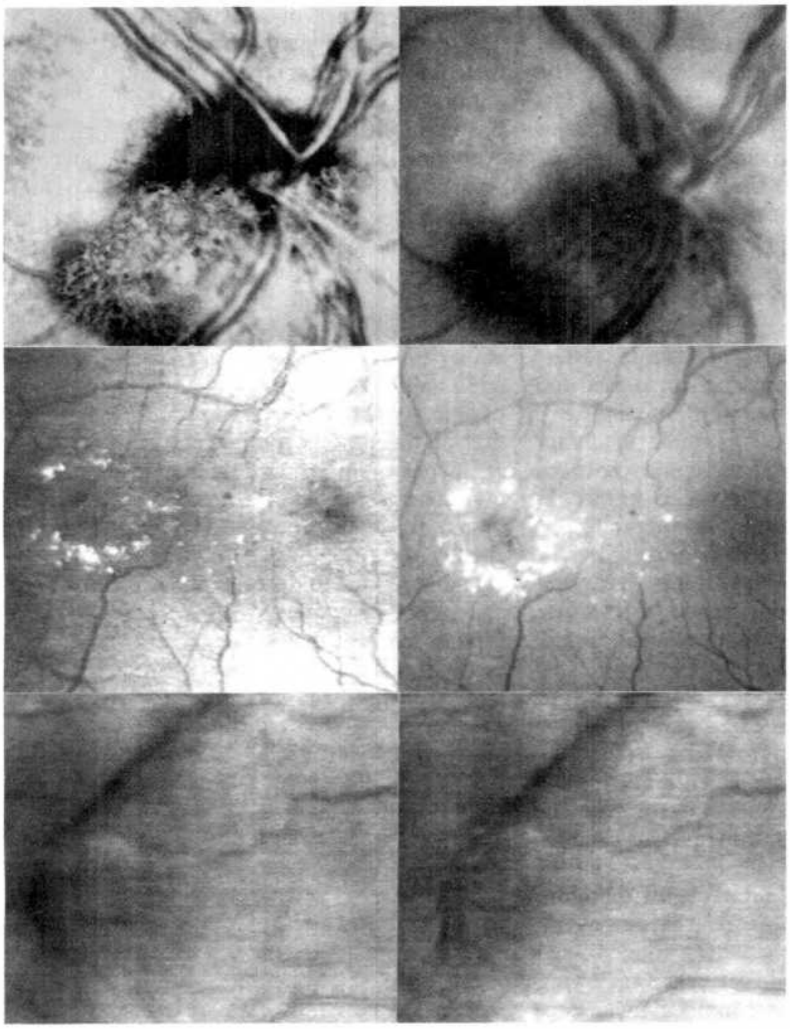

Figure 6 Top: confocal (left) and fundus camera (right) image of a disc melanocytoma. The contrast of structures within the melanocytoma is increased in the confocal image. Centre. confocal (left) and fundus camera (right) image of diabetic exudates taken on the same day. The exudates appear to be more extensive in the fundus camera image. Bottom: confocal images, taken a short time apart, of a vitreous discontinuity in a patient with low tension glaucoma. The vitreous discontinuity appears to be fixed at the macula.

ing the functions of a fundus camera. The fundus camera produces an image that is equivalent to that observed by the clinician using an ophthalmoscope. The confocal image produced by the cSLO changes the appearance and visibility of structures from that in a conventional image. This allows some, but not all, structures to be better seen in a confocal image.

The scanning laser ophthalmoscope has been developed to image the fundus without the need for mydriatics.[3] This would be an advantage when there is a large through-put of patients such as in screening programmes. In the cSLO, this advantage was only consistently found when using the 40 degree field of view. Using the 20 degree field of view, a mydriatic was found to be helpful. This is due to the increase in laser beam diameter at the pupil, from 1 mm to 2 mm, when changing from the 40 degree to the 20 degree field of view.[10] Thus, in the 20 degree field of view mode, a larger area of the pupil is used for the illumination and a pupil size greater than 2 mm is required to obtain a view of the fundus. The 20 degree field of view mode gives a more confocal image, and in this study the minor inconvenience of using a mydriatic was accepted in order to produce a more confocal image. The cSLO has been shown to give a degree of optical sectioning. The best axial resolution of the cSLO was found to be 300 μm (FWHM) in a model eye. This agrees with the results of other investigators.[10] The optical sectioning demonstrated in the live eye was probably of the same order and would be sufficient to section the retinal features from the lamina cribrosa in patients with deep cups. However, most other clinically important features in the fundus lie within too narrow a plane for them to be optically sectioned from each other. Although an axial resolution of 300 μm is disappointing in terms of optical sectioning of the fundus, it does improve contrast by avoiding flare from the ocular media. Contrast is also improved in the confocal mode by avoid-

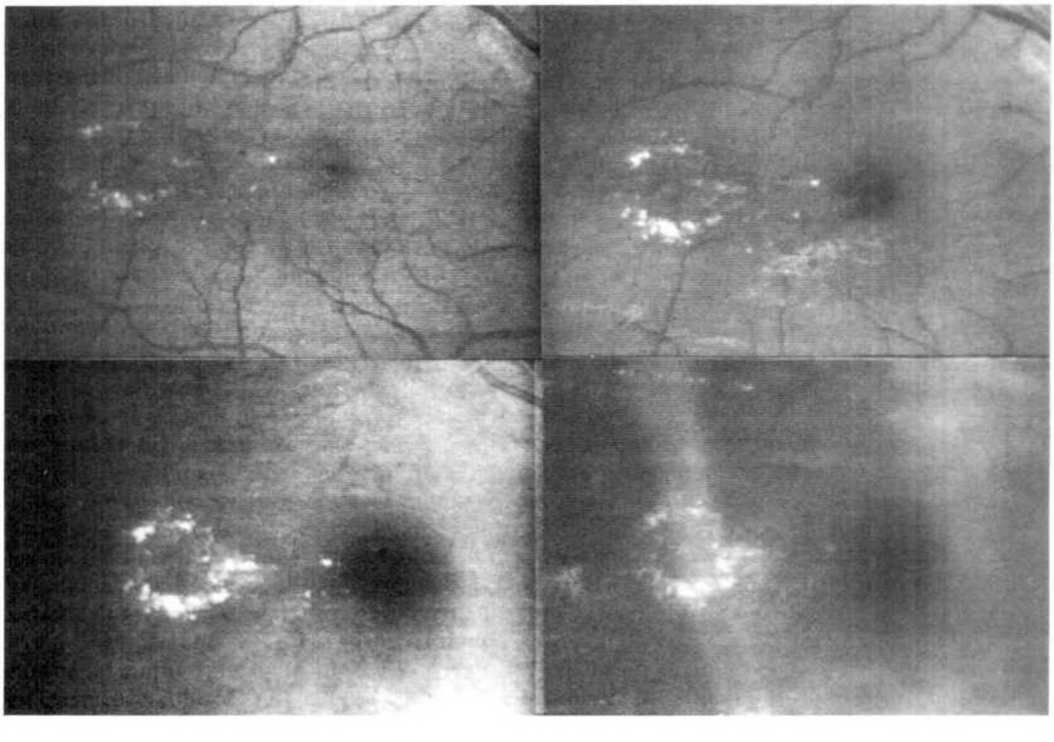

Figure 7 SLO images of a patient with diabetic maculopathy taken using the 40 degree field of view. Top left: confocal aperture 3. Top right: confocal aperture 1. Bottom left: dark field image. Bottom right: non-confocal image. The blood vessels are seen in higher contrast in the confocal images while the exudates are less visible in the confocal images.

ing multiply scattered light from the fundus. It was found that the improved contrast of confocal imaging is of particular value in improving the resolution of structures that are seen in low contrast in conventional fundus camera images (Figs 5 and 6). The avoidance of light scattered from the nerve fibres in the optic nerve head allowed optic nerve head drusen to be imaged as discrete nodules. Similarly in two patients, it was possible to image the lamina cribrosa underlying the neuroretinal rim. This was probably due to a relatively thin neuroretinal rim in these two patients, while in the majority of patients scattering of light by the neuroretinal rim caused too much attenuation of the laser illumination. For similar reasons, it was not possible to obtain multiple optical sections of the lamina cribrosa even though the lamina cribrosa is known to extend over 1 mm. Nonetheless, the ability to image the surface of the lamina cribrosa will be of benefit in examining the hypothesis that optic nerve head damage from raised intraocular pressure is related to the structure of the lamina cribrosa.[11 12]

The improved contrast of the confocal images is gained at the cost of the visibility of structures which multiply scatter light. These structures are well imaged using a conventional fundus camera, and this demonstrates the complementary nature of the cSLO and the fundus camera. Hard exudates are clearly visible in the dark field mode of the cSLO (Fig 7). They must therefore multiply scatter light. Their apparent extent in a confocal image is less than in a conventional image, and it is important that, when reviewing patients with hard exudates, confocal images are not confused with conventional images. However it would be reasonable to compare directly an SLO image produced using the non-confocal mode with a conventional fundus camera image.

The cSLO was found to image vitreous discontinuities in higher contrast than a fundus camera. The vitreous disturbances were usually seen as shadows floating across the screen. However, in one patient, the vitreous was found to be attached around the fovea (Fig 6, bottom). The dynamic image from the cSLO showed movement of the area of abnormal vitreous with one end remaining attached around the fovea. Further observations on vitreous attachments using the cSLO may help to clarify the role of the vitreous in the development of macular holes.[13]

The subjective quality of the confocal images was good for the dynamic cSLO image, while single frame images captured from the dynamic image contained noise that was not evident in the dynamic image. This difference is probably due to the ability of the human brain to average over successive frames of a dynamic cSLO image. The quality of a dynamic image could be approximated by computer averaging over 16 frames with manual alignment of the individual frames. No extra details were obtained by computer averaging that could not be seen in the dynamic recording. However it is possible that more accurate alignment of the images by computer or other forms of computer enhancement could provide greater resolution of details of the fundus.

The cSLO has created most interest by its ability to produce high quality dynamic fluorescein angiograms and by its ability to perform pyschophysical tests with simultaneous visualisation of the fundus. Our results show that the cSLO has an important role to play in providing high contrast images of structures that are seen in low contrast using conventional techniques. The cSLO, by providing confocal imaging, is a valuable adjunct for imaging the fundus. Further experience of confocal imaging of pathological fundi is required to evaluate fully the potential of confocal imaging for clinical use. However it is expected that confocal imaging will make a valuable contribution to clinical research.

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The effects of subconjunctival betamethasone on the blood aqueous barrier following cataract surgery: a double-blind randomised prospective study

S M Shah, J D McHugh, D J Spalton

Abstract

The aim of this double-blind randomised prospective study was to assess the effect of subconjunctival Betnesol (betamethasone sodium phosphate 0·1%) on the recovery of the blood aqueous barrier (BAB) following cataract surgery in uncomplicated eyes. Twenty patients [10 male, mean age 71·4 (SD 12·7) years] admitted for routine cataract surgery were randomised into two groups. All patients recruited into the study were free of other ocular disease and were not taking any anti-inflammatory medication. Group A received a subconjunctival injection of cefuroxime (125 mg) alone while group B received a subconjunctival injection of both cefuroxime and Betnesol. All surgery was performed by a single surgeon using a standardised surgical technique and all patients received the same postoperative medication. The Kowa laser flare cell meter was used to measure aqueous flare and cells preoperatively and on the first, second, and seventh postoperative day, and at 1 and 3 months following surgery. The code was broken only after all patients had been followed-up for 3 months postoperatively. There was no significant difference between the two groups in aqueous flare and cells at any of the postoperative visits. In this study we were unable to demonstrate any beneficial effect of subconjunctival betamethasone on damage to and recovery of the BAB following cataract surgery in the uncomplicated eye.

(*Br J Ophthalmol* 1992; 72: 475–478)

Many surgeons give an injection of subconjunctival steroids at the end of a cataract operation in the belief that it will produce a quieter eye postoperatively, with less damage to the blood aqueous barrier (BAB), a more rapid recovery of the eye, and a reduction in postoperative inflammatory complications. However, to the best of our knowledge, this has never been clinically proved. At present therefore some surgeons use subconjunctival steroids routinely in all cases, some not at all, and others use them only in selected high risk cases. The aim of this double-blind randomised prospective study was to assess the role of subconjunctival steroids in cataract surgery in the normal eye. Until recently the objective assessment of damage to the BAB has only been possible using anterior segment fluorophotometry.[1] However this technique is invasive, time-consuming, and the results are often difficult to interpret. The laser flare cell meter is a recently introduced instrument that allows a rapid and non-invasive quantification of aqueous flare and cells by measurement of light scattering of a helium-neon (He-Ne) beam in the anterior chamber.[2] The instrument has been shown to be highly accurate and reproducible[3] and to compare favourably with fluorophotometry in assessment of damage to the BAB following cataract surgery.[4] We have used this instrument to document objectively the effect of subconjunctival betamethasone (Betnesol) on postoperative flare and cells.

Medical Eye Unit, St Thomas' Hospital, London
S M Shah
J D McHugh
D J Spalton

Correspondence to:
D J Spalton, The Medical Eye Unit, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH.

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Materials and methods

THE LASER FLARE CELL METER

The laser flare cell meter (Kowa FC 1000) consists of an He-Ne laser slit-lamp, a binocular microscope fitted with a photomultiplier and a personal computer.[1] The He-Ne beam has a power of 25 μW and a diameter of 20 μm. The beam is projected into the anterior chamber and scattering of the beam within a sampling window ($0·3\times0·5$ mm) is detected by the photomultiplier. Two measurement modes are used, one for protein concentration and the other for cell count. The instrument automatically converts from one mode to the other. Each mode takes 0·5 seconds and thus the total measurement time is 1 second. Computer analysis distinguishes between photons reflected from cells or protein. In the protein concentration measurement mode the laser beam is scanned vertically for a length of 0·6 mm, covering the sampling window. Scattering of this beam is measured when it passes through this window. Measurements are also taken when the beam passes above and below the sampling window to assess the background signal (BG1 and BG2). The flare value in photon counts/ms is calculated by subtracting the signal value from a mean of two background counts. In the cell count mode the beam is scanned two dimensionally ($0·6\times0·25$ mm) in the sampling window and the scanning volume corresponds to 0·075 mm^3. A strong peak of scattered light is obtained when the beam strikes a floating cell, and the number of peaks is counted by the computer.

Scans in which the difference between BG1 and BG2 exceeded 15% (indicative of a non-uniform background) were excluded and the scan repeated. To prevent 'massaging' of data, once the instrument had been aligned each sequential scan was accepted provided the difference between the background counts was less than 15%. The results of five scans were then averaged. Laser flare cell measurements were performed preoperatively, and on the first, second, and seventh postoperative day and at 1 and 3 months following surgery.

SUBJECTS

Informed consent was obtained from all patients prior to the study. Twenty patients admitted to St Thomas' Hospital for routine cataract surgery were recruited for the study. All patients entered into the study were free of other ocular disease and were not taking any anti-inflammatory medication. Patients with systemic diseases known to affect the BAB (for example, diabetes) were also excluded from the study. The patients were then randomised on a double-blind basis into two groups (groups A and B). Patients in group A were allocated to receive a subconjunctival injection of cefuroxime 125 mg alone while patients in group B were to receive a subconjunctival injection of both cefuroxime 125 mg and Betnesol (4 mg). The basis of allocation was known only to the surgeon, the code being broken after a 3 month follow-up had been completed on all patients. The surgeon was not involved in the postoperative assessment of the patients.

SURGERY

All surgery was performed by the same surgeon (JDM) using a standardised extracapsular surgical technique. At the completion of the procedure the surgeon was given the patient's code (group A or B) and the appropriate subconjunctival injection administered. Subconjunctival injections were administered transconjunctivally into the inferotemporal fornix. Postoperatively all patients received the same medication consisting of topical Maxitrol (dexamethasone 0·1% and neomycin 0·35%) four times daily for the first week and twice daily for a further 3 weeks.

STATISTICAL ANALYSIS

Differences between the two groups in the distribution of race, sex, and iris colour were analysed using the χ^2 test. Laser flare and cell data were not normally distributed even after logarithmic transformation. Therefore differences in aqueous flare and cells between the two groups were analysed using the non-parametric Mann-Whitney test; p values of less than 0·05 were considered to be statistically significant. All variables are presented as the mean (1 SD).

Table 1 Characteristics of patients in groups A (no subconjunctival betamethasone) and B (subconjunctival betamethasone)

	Group A	*Group B*	*p Value*
Age (years)	69·2±15·7	73·6±7·5	0·67
Sex male	5/10	5/10	0·65
Race Caucasian	9/10	9/10	0·46
Iris colour brown	4/10	3/10	1·0

The mean ages (SD) and distribution of sex, race, and iris colour in patients in groups A and B. (Group A: no subconjunctival betamethasone, group B: subconjunctival betamethasone.) The two groups were well matched for all these characteristics.

Table 2 Postoperative visual acuity

Visual acuity	*Group A* (n=*10*)	*Group B* (n=*10*)
6/5	1	0
6/6	2	6
6/9	4	3
6/12	2	1
6/18	1	0

The best corrected postoperative visual acuity at 3 months in groups A and B. (Group A: no subconjunctival betamethasone, group B: subconjunctival betamethasone).

Results

Twenty patients (10 male, 18 Caucasian, mean age 71·4 (SD 12·7) years, range 34–90 years) were randomised to receive either a subconjunctival injection of cefuroxime and Betnesol or cefuroxime alone. The two groups were well matched in their distribution of age, sex, race, and iris colour (Table 1). There was no significant difference (p=0·35) between the two groups in the distribution of best corrected visual acuity at 3 months following surgery with over 90% of patients in each group achieving a visual acuity of 6/12 or better (Table 2).

The changes in aqueous flare and cells following surgery are shown in Figure 1. There was a peak in aqueous flare and cells on the first postoperative day, followed by a rapid decline in the first week, with a more gradual return to normal levels by 3 months following surgery. There was no significant difference (p>0·05) between the two groups in both flare and cells at any of the postoperative visits. On the first postoperative day there was also no difference between the two groups in the number of eyes

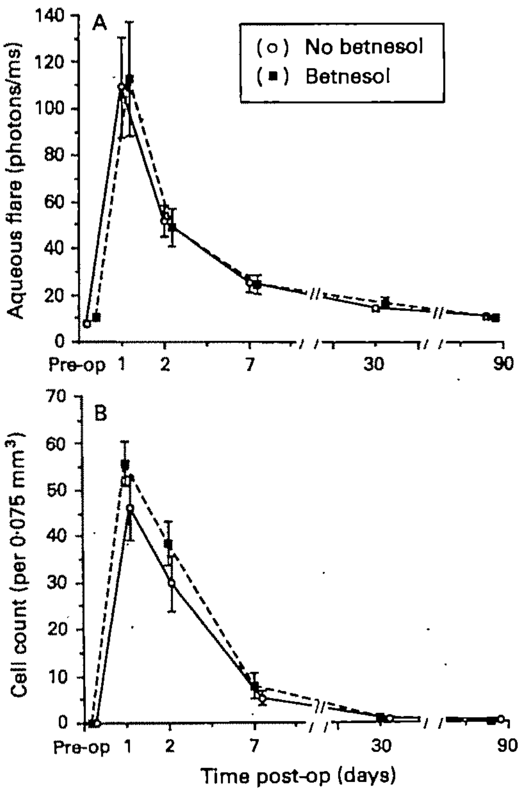

Figure 1 Changes in aqueous flare and cells following cataract surgery. Line plot of mean (SEM) aqueous flare (A) and cells (B) against days following surgery for groups A and B. (Group A: no subconjunctival betamethasone, group B: subconjunctival betamethasone.)

(three eyes in each group) with marked anterior chamber inflammation (flare values above 150 photons/ms). There were no cases of postoperative iritis in either group requiring a change in the standard topical medication regimen.

Discussion

In this preliminary study we have shown that subconjunctival betamethasone has no beneficial effect on damage to the BAB (as measured by the laser flare cell meter) following cataract surgery in uncomplicated eyes. In view of the proved effect of topical steroids given later on in the postoperative period, the most likely reason for this finding is that the effect of subconjunctival Betnesol is short lived and complete within too short a time to influence the later inflammatory consequences of surgery.

Most of the published reports on the kinetics of subconjunctival injections are based on animal studies. Maurice and Ota[5] found that following a subconjunctival injection of small volumes of tracers (^{22}Na, ^{131}I-hippurate, albumin) in the rabbit eye there was rapid loss of the tracer from the subconjunctival space into the blood. The half-life varied from 9 minutes for ^{22}Na and 20 minutes for ^{131}I-hippurate to 9 hours for albumin. After 1 hour ^{22}Na was no longer detectable at the injection site and there was little penetration of tracer into the anterior chamber. In the case of ^{22}Na the peak concentration in the aqueous occurred within the first hour, and was only 1% of the total injected dose. With the larger molecules the peak concentration occurred later and also at a lower percentage of total dose. They concluded that penetration into the aqueous was only possible for a brief period after injection due to the rapid clearance of tracer from the injection site into blood. Wine *et al*,[6] also using the rabbit, investigated the time course and route of ocular penetration of radio-labelled ^{14}C hydrocortisone from a subconjunctival injection (0·25 ml of a 2·5% solution). They found that a peak ocular concentration of hydrocortisone (176·7 μg) occurred within 30 minutes of injection with a rapid decline in concentration within 2 hours (41·1 μg) and a more gradual decline to 1·8 μg at 72 hours. If the technique of subconjunctival injection was modified so that the conjunctival surface remained intact the peak concentration was significantly reduced (3·9 μg). They concluded that the major route of ocular penetration from a subconjunctival injection of hydrocortisone was therefore by leakage through the needle puncture wound into the conjunctival sac and then through the cornea to the anterior chamber by the tear film. However autoradiographic studies in rabbit eyes do indicate that subconjunctival hydrocortisone can penetrate directly through the sclera from the injection depot site. McCartney *et al*[7] found that penetration occurred within 30 minutes in the inflamed eye and only after 4 hours in the normal eye. They also confirmed that only a very small proportion of the drug penetrated into the eye (about 1–2%).

It has been assumed in most of the animal studies on this subject that the results are relevant and applicable to humans. However Maurice and Ota[5] have found marked differences between the rabbit and human eye in the penetration of fluorescein into aqueous from subconjunctival injections, and they have therefore questioned the validity and relevance of results obtained in the rabbit model. There are few reports on the kinetics of subconjunctival injections in humans. They have been carried out on normal eyes and may not reflect the pharmacokinetics in inflamed or postoperative eyes where changes in aqueous flow or leakage may be important. Jain *et al*[8] measured the concentration of both hydrocortisone acetate and dexamethasone sodium phosphate in aqueous humour in normal eyes following subconjunctival injection (dexamethasone 0·5 ml of 0·4% solution, hydrocortisone 0·5 ml of 2·5% solution). Peak concentration occurred within 10 minutes; dexamethasone was no longer detectable at 48 hours after injection and although hydrocortisone was detectable at 7 days after injection it was only present in very low concentrations. It would appear from these studies that the penetration of a solution of steroids into aqueous humour from a subconjunctival site is often poor. No information is available about what tissue levels of steroid are effective and a potential therapeutic effect may be over within hours. If postoperative inflammation depends on recruitment of inflammatory cells this may occur too late to be influenced by a perioperative injection. Mechanical trauma at the time of surgery may be the main contributing factor to the extent of damage to the BAB on the first postoperative day.[9] Therefore the use of a short-acting anti-inflammatory agent immediately after surgery may be relatively less important than it is in the subsequent days following surgery when a true immunological inflammatory response is likely to be more significant. This would be consistent with clinical studies in which postoperative iritis and particularly fibrinous iritis is reported to occur usually 3–5 days after surgery.[10 11]

The absorption of subconjunctival steroids and their therapeutic effect is also affected by the type of steroid used and the vehicle used to deliver it. Betamethasone and dexamethasone have 5–7 times greater systemic anti-inflammatory activity than prednisolone on a weight for weight basis. The absorption of these steroids into aqueous following topical application has been shown to be significantly different depending on the vehicle used. McGhee *et al*[12] measured the aqueous concentrations (using gas chromatography with mass spectrometry (GCMS)) of prednisolone acetate (1%), fluoromethalone alcohol (0·1%), dexamethasone alcohol (0·1%), betamethasone sodium phosphate (0·1%), and prednisolone sodium phosphate (0·5%) following single drop instillation. They found a large variation in peak aqueous concentration, ranging from 669·6 ng/ml with prednisolone acetate, 31·0 ng/ml with dexamethasone alcohol, 25·6 ng/ml with prednisolone sodium phosphate, and 7·7 ng/ml with betamethasone sodium phosphate. Acetate and alcohol steroid preparations are lipophilic and penetrate the corneal epithelium better than polar preparations such as sodium salts of the steroid phosphate. The appropriately adjusted index of concentration for these steroids (assuming a seven-fold difference in potency between prednisolone and the other steroids) are 95·7 for prednisolone acetate, 31·0 for dexamethasone alcohol, 7·7 for betamethasone sodium phosphate, and 3·3 for prednisolone sodium phosphate. Therefore the use of subconjunctival betamethasone in its sodium phosphate form may also theoretically reduce its absorption and minimise the therapeutic effect. Other types of steroid, more long acting or reservoir preparations may be better suited to perioperative use.

In conclusion we have found in this preliminary study that the use of subconjunctival betamethasone has no beneficial effect on damage to and recovery of the blood aqueous barrier following cataract surgery in otherwise normal eyes. The incidence of postoperative inflammatory complications is greater in diabetics and in eyes with glaucoma and uveitis and similar further studies are required to assess both the role and type of subconjunctival steroids in these high risk groups. We therefore feel that at present the routine use of subconjunctival steroids in cataract surgery is not cost-effective and should be restricted to high risk cases and in those patients who develop operative complications.

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Long-term survival of endothelium following transplantation of corneas stored by organ culture

R M Redmond, W J Armitage, J Whittle, S J Moss, D L Easty

Abstract

This study reports corneal graft survival, endothelial cell changes, and visual outcome in 20 patients who received some of the first corneas stored by organ culture in the Corneal Transplant Service Eye Bank in Bristol. Mean donor age was 48 years (SD 15, *n*=20) and corneas were stored for an average of 21 days (SD 7, *n*=20). Preoperative endothelial cell density was 2334 cells/mm² (SD 235, *n*=18) and this fell by 8% (SD 12) to 2158 cells/mm² (SD 372) within the first 2 months following transplantation. In 13 patients, endothelial cell density thereafter declined exponentially with a half-life of 41 months (SD 17, *n*=12; one patient excluded as an outlier). Corneas that suffered rejection episodes showed the highest rates of loss of endothelial cells. Endothelial cell loss 4 years after transplantation was 46% (SD 16, *n*=12), which was similar to the postoperative decline in cell density reported for corneas stored for far shorter periods in McCarey-Kaufman medium at 4°C.

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The Corneal Transplant Service (CTS) Eye Bank was set up in Bristol in 1986 to help meet the increasing demand for corneal tissue in the UK.[1 2] Organ culture at 34°C was chosen as the principal storage method because it offered significant advantages.[3-5] Organ culture allows corneas to be stored for more than 4 weeks, facilitating elective transplantation, reducing tissue wastage and improving the distribution of corneas from tissue-typed donors. Bacterial or fungal contamination of donor tissue is minimised by cleaning eyes before excision of corneoscleral discs and by the addition of antibiotics and antimycotics to the culture medium. Finally, Coster[6] has speculated that organ-cultured corneas might be less antigenic than corneas stored at 4°C.

Since 1986, more than 6500 corneas stored in the CTS Eye Bank have been supplied to hospitals throughout the UK. Although short-term outcome of organ-cultured grafts has been reported,[7 8] Galloway[9] has referred to the lack of long-term follow-up. The aim of the present study was to follow the long-term endothelial changes in corneal grafts in patients who received some of the first corneas stored by organ culture in the CTS Eye Bank.

Methods

Patients

Twenty patients who had undergone penetrating keratoplasty in Bristol Eye Hospital using corneal tissue stored by organ culture were followed for up to 4 years. One patient died in the first year and was lost to follow-up. Mean patient age was 56 years (SD 20, *n*=20) and there was a preponderance of females to males of 13:7. There were five cases of Fuchs' endothelial dystrophy, three cases of herpes simplex keratitis, three regrafts (original diagnoses were herpes simplex keratitis and, in two cases, keratoconus), three cases of bullous keratopathy, two cases of keratoconus, two cases of lattice dystrophy, and two cases of interstitial keratitis.

Corneal grafts were performed according to a standard procedure at Bristol Eye Hospital. Donor buttons were punched from the endothelial side using Pharmacia disposable trephines that were 0·25 mm larger than those used for the recipient. A double continuous suture was used and the knots were buried at the interface. Particular care was taken to ensure a watertight interface.

Donor corneas

Mean donor age was 48 years (SD 15, *n*=20) and the corneas were stored for an average of 21 days (SD 7). Corneas were stored at 34°C in Eagle's minimum essential medium containing hepes buffer, fetal calf serum (2%), sodium bicarbonate (24 mmol/l), L-glutamine (2 mmol/l), penicillin (100 units/ml), streptomycin (0·1 mg/ml), and amphotericin B (0·25 μg/ml).[5] Two days before transplantation, corneas were removed from the culture medium and the endothelium was examined using a light microscope following staining with trypan blue and sucrose. This allowed corneas with fewer than 2000 endothelial cells/mm² or with endothelial defects to be excluded. The endothelium was also photographed to permit a more accurate determination of preoperative cell density. Corneas were then placed into medium containing 5% dextran and returned to 34°C for a further 2 days to thin them prior to transplantation.

Postoperative endothelial cell density

Patients were examined with a wide-field specular microscope (Keeler-Pocklington) at 2, 6, and 12 months following transplantation and then at yearly intervals. Follow-up was not available for every patient at all follow-up times. Scanning of the posterior corneal surface concentrated on the central 4-mm region and did not follow a rigid grid pattern.[10] Pachymetry was performed using the Pocklington microscope during the first postoperative year and intraocular pressure was monitored using Goldmann applanation tonometry throughout the 4-year follow-up period.

Department of Ophthalmology, Bristol University
R M Redmond*
W J Armitage
J Whittle
S J Moss
D L Easty

Correspondence to:
Dr W J Armitage, Department of Ophthalmology, Bristol Eye Hospital, Lower Maudlin Street, Bristol BS1 2LX.

Present address: Moorfields Eye Hospital, City Road, London EC1V 2BD.

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Endothelial morphometric analysis was carried out using 35-mm negatives and a Kontron-MOP system. A 0·01 mm² grid in the film plane of the specular microscope ensured that each 35-mm negative could be scaled accurately. Measurements of cell perimeter, cell surface area, maximum diameter, and shape factor were made on up to 50 individual cells from each cornea. Cell clusters were avoided and cells were selected at random from 5–6 negatives. Cell density was calculated from the reciprocal of cell area. Thus, up to 50 estimates of cell density were obtained for each patient at any given postoperative assessment time.

Changes in endothelial cell density with time were studied in individual patients. An exponential equation of the form $D=D_0e^{-kt}$, where D is cell density (cells/mm²), D_0 is initial cell density, k is an exponential rate constant and t is time after transplantation (months), was fitted to the data from each patient by least squares regression, ensuring equal numbers of estimates of cell density at each follow-up time. The time taken for cell density to fall by 50% (the half-life) was calculated for each patient, where appropriate, from $0{\cdot}693/k$.

Results

Clinical outcome

Immediately following transplantation, organ-cultured corneas were thicker and more opalescent than corneas that had been stored at 4°C. The inflammatory response of the anterior segment was low, but grafts cleared relatively slowly, taking 3–4 weeks to achieve good transparency. Six months after transplantation, mean corneal thickness was 0·53 mm (SD 0·06, $n=9$) and intraocular pressure was 17·2 mm Hg (SD 6·0, $n=9$).

Fine folds in Descemet's membrane were universal and persisted adjacent to the graft-host interface. These folds did not impair vision because the central area was quite clear after the first month. The improvement in visual acuity, excluding those cases where retrocorneal pathology precluded good macular function, is shown in Figure 1. Astigmatism at 1 year ranged from 0–9 dioptres with a mean value of 4·1 (SD 2·7, $n=13$).

Endothelial changes

Preoperative cell density of the donor corneas was 2334 cells/mm² (SD 235, $n=18$ – data not available for two corneas). Two months after transplantation, endothelial cell density in this same group of patients had declined to 2158 cells/mm² (SD 372, $n=18$: $p<0{\cdot}01$, *cf* pre-op density, paired *t* test). This represented an average loss of 8% (95% CI 2–14%, $n=18$). Follow-up data for 4 years was available for 12 patients (Table 1). Four years after transplantation, these corneas had lost 46% (95% CI 35–56%, $n=12$) of their cells compared with the preoperative cell density. The cell loss compared with the 2-month postoperative cell density was 42% (95% CI 30–53%, $n=12$).

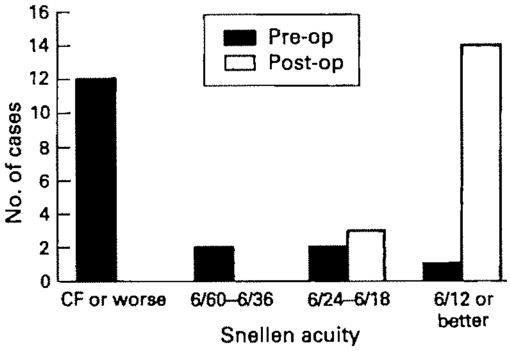

Figure 1 Change in visual acuity following transplantation.

After the initial fall during the first 2 postoperative months, endothelial cell density continued to decline exponentially in 13 of the transplanted corneas. The other seven corneal grafts showed inconsistent patterns of change in cell density – there were no overt reasons for this difference (Table 2). Because exponential rates of cell loss varied significantly between patients (p<0·001), the data were not pooled to calculate a single regression equation; however, the mean half-life for the decline in cell density was 41 months (SD 17, $n=12$). One cornea with a half-life of 407 months was excluded as an outlier (p<0·01, Dixon test). Two corneas that suffered rejection episodes and subsequently failed after 3 years had the shortest half-lives (that is, the highest rates of cell loss), namely 14·7 and 21·5 months. Of the 12 corneas with 4-year follow-up, seven (excluding the outlier) showed an exponential decline in cell density with an average half-live of 47 months (SD 16, $n=7$).

Discussion

Bourne *et al*[7] suggested that survival of donor endothelial cells following transplantation is a

Table 1 Change in mean endothelial cell density of corneal grafts in 12 patients followed for 4 years

	Cells/mm² (SD)
Preoperative cell density	2379 (242)
Postoperative cell density:	
2 months	2233 (380)
48 months	1292 (375)

Table 2 Comparison between the group that showed an exponential postoperative decline in endothelial cell density and the group that showed no consistent pattern of change

	Exponential (n=*13*)	*Inconsistent* (n=7)
Patient:		
Age (years)	51 (SD 21)	64 (SD 18)
Diagnosis:		
Fuchs' dystrophy	4	1
Regraft	2	1
Keratoconus	2	–
Herpes keratitis	1	2
Lattice dystrophy	2	–
Interstitial keratitis	1	1
Bullous keratopathy	1	2
Donor cornea:		
Donor age (years)	49 (SD 14)	46 (SD 18)
Storage time (days)	21 (SD 8)	22 (SD 7)
Pre-op cells/mm²	2320 (SD 188) ($n=11$)*	2357 (SD 311)
2-month cell/mm²	2147 (SD 226) ($n=11$)	2175 (SD 554)
2-month cell loss (%)	7 (SD 8) ($n=11$)	8 (SD 16)

*Preoperative cell densities were not available for two corneas in the exponential group.

more sensitive indicator of the efficacy of corneal preservation techniques than clinical outcome because corneas can remain transparent with as few as 500 cells/mm^2.[11] Bourne and his colleagues have reported long-term changes in transplanted corneas following storage in McCarey-Kaufman (M-K) medium at 4°C[7,8] and short-term changes in corneas stored in organ culture at 34°C.[12,13] Their data thus form a baseline for comparison with the changes observed in the present study.

Preoperative cell densities were higher in Bourne's studies,[7,8,12,13] ranging from approximately 2800–3300 cells/mm^2, than the 2334 cells/mm^2 (95% CI 2217–2451, $n=18$) of the present study. However, the initial loss of cells during the first 2 months following transplantation in the two long-term studies of corneas that had been stored in M-K medium[12,13] was far higher (>30%) than the 8% (95% CI 2–14%, $n=18$) seen in the Bristol patients. Thereafter, the loss of cells betwen 2 and 48 months of approximately 45% reported by Bourne[12] and by Matsuda and Bourne[13] was similar to this study – namely, 42% (95% CI 30–53%, $n=12$).

Although based on mean cell densities calculated from pooled data rather than on the changes observed in individual patients, Bourne[7] also found an exponential decline in cell density, but only over the first 3 years after transplantation. The slope of his regression equation gives a half-life of 2·9 years, which is similar to the 3·4 years (95% CI 2·5–4·3 years, $n=12$) observed in the Bristol patients.

In another study, the efficacy of organ culture was rightly called into question by Bourne *et al*[12] who reported higher rates of endothelial cell loss over the first 2 postoperative months from organ cultured corneas (28%, SD 17, $n=75$) than from corneas stored in M-K medium (9%, SD 17, $n=75$). One year after transplantation, the cell density of corneas stored in M-K medium was 2121 cells/mm^2 (SD 680, $n=47$) compared with 1704 cells/mm^2 (SD 702, $n=47$) in organ cultured grafts, representing cell losses of 25% (SD 24, $n=47$) and 44% (SD 19, $n=47$), respectively. The initial cell loss from corneas stored in M-K medium was much lower than in Bourne's earlier series[12,13] and is similar to the loss seen in the present series of organ-cultured grafts. Furthermore, despite a lower preoperative cell density, the Bristol organ-cultured grafts had a 1 year cell density of 1946 cells/mm^2 (95% CI 1537–2356, $n=12$).

Bourne *et al*[7] showed that transferring corneas from organ culture to M-K medium at 4°C for several hours before transplantation, presumably to thin the corneas, was deleterious. They pointed out that the temperature change from 34° to 4°C might compromise the ability of the endothelium to survive the trauma of transplantation. Corneas that were organ cultured but not transferred to M-K medium at 4°C had lower rates of cell loss, although the rates were still higher than in corneas stored only in M-K medium. The organ culture technique used in Bristol was orginally developed in Århus and Amsterdam and differs from that used formerly in Minneapolis. Two days before transplantation, the corneal endothelium is examined and, if judged to be suitable, the cornea is placed into medium containing 5% dextran, but the cornea is not refrigerated. This allows the cornea to dehydrate and thin prior to transplantation. The corneas transplanted by Bourne *et al*[12] were either refrigerated or were thickened at the time of transplantation. The inclusion of 1·35% chondroitin sulphate in the organ culture medium eliminated the difference in cell loss between organ culture and M-K storage observed by Bourne *et al*[7,8] which, taken together with the present study, suggests that intermediate-term organ culture can be as effective as short-term 4°C storage at preserving the endothelium.

In summary, this study of patients grafted in Bristol suggests that the rate of postoperative endothelial cell loss from organ cultured corneas is no worse than the loss expected from corneas stored for much shorter periods at 4°C, thus confirming the efficacy of intermediate-term corneal storage by organ culture. In the early postoperative period, eyes that received organ-cultured corneal grafts were judged to be 'quiet', and problems with severe anterior uveitis were not encountered. This may have been related to the elimination of bacterial or fungal contamination during organ culture or to a putative reduction in antigenicity.[6]

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Assessment of central visual function after successful retinal detachment surgery by pattern visual evoked cortical potentials

Masahiro Ueda, Emiko Adachi-Usami

Department of Ophthalmology, School of Medicine, Chiba University, Chiba, Japan
M Ueda
E Adachi-Usami

Correspondence to:
Dr Masahiro Ueda,
Department of Ophthalmology, School of Medicine, Chiba University,
Inohana 1-8-1, 280 Chiba,
Japan.

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Abstract

The pattern of visual recovery after successful surgery by pattern visual evoked cortical potentials (VECP), visual acuity, colour vision, and critical fusion frequency was investigated in 14 eyes with retinal detachment involving the macula. The temporal tuning characteristics in the evoked potentials were measured as based on the P_{100} amplitude and the frequency necessary for evoking 0 μV amplitude, which was defined as an objective critical fusion frequency by extrapolating the curve. Significant improvement in visual acuity and colour vision was observed within 2 months postoperatively. A significantly increased P_{100} peak latency became shorter as the postoperative days increased. In general, a good correlation was noted between the P_{100} peak latency and subjectively measured visual acuity, colour vision, and critical fusion frequency. The objective critical fusion frequency measured by VECP recovered gradually during the 6 months after surgery. Functional recovery was not related to the length of time the macula was detached before surgery.

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A discrepancy exists between anatomical recovery after retinal detachment surgery and subjective visual functions as represented by visual acuity and colour vision. Several reports[1-4] on subjective visual functions after successful retinal detachment surgery have indicated that, although visual function improved gradually within several months after operation, it never returned to the preoperative level. The influencing factor is believed to be duration of macular detachment.[4-12]

Two methods of measuring central visual function objectively have recently been developed. One is focal macular electroretinography and the other is visual evoked cortical potentials (VECP) to pattern stimulation. The latter reflects central visual function,[13] without using the complicated retinal stimulus conditions necessary for a local electroretinogram. Surprisingly little is known from the electrophysiological standpoint, particularly using pattern VECPs after retinal detachment surgery.

We evaluated central visual function objectively with the use of pattern VECPs and subjectively with visual acuity, colour vision, and critical fusion frequency in patients with rhegmatogenous retinal detachment involving the macula who underwent successful retinal repair.

Subjects and methods

Fourteen patients with unilateral rhegmatogenous retinal detachment involving the macula, who had successful anatomical surgical results showing no macular pucker or cystoid macular oedema between December 1989 and July 1990 at our clinic, have been followed-up for 6 months. All subjects who participated in this study were fully informed of the procedures and gave their consent for inclusion. The nine male and five female patients ranged in age from 14 to 65 years (mean age 38·5 years). Of the 14 patients, 13 eyes underwent conventional surgical techniques of scleral buckling with cryopexy and drainage of fluid; one eye was treated with pneumatic retinopexy and light coagulation. The duration of preoperative macular detachment, which was estimated as the time from onset of subjective visual loss to the day of surgery, ranged from 10 to 16 days for nine eyes. Excluding the case representing the longest duration (6 months), the mean duration was 17·8 days. In all cases preoperative visual acuity was 0·2 or less, ranging from 0·01 to 0·2 (mean 0·09).

Visual acuity, colour vision, critical fusion frequency, and pattern VECPs were examined at 2 weeks and 1, 2, 4, and 6 months after surgery. The fellow eyes were tested as controls. The data were statistically analysed, based on student's *t* test.

Visual acuity was corrected for refraction with use of the autorefractometer (RK-1, Canon Ltd, Japan) and skiaskopy after administration of atropine. With the appropriate near correction, colour tests were administered monocularly under a level of 800 lux at the test plane given by a 20 W Toshiba EDL-65 fluorescent tube.

The standard pseudochromatic plates, part II, and panel D-15 were used for tests of colour vision. During the follow-up period, all patients passed a panel D-15 test. The results of the pseudochromatic test showed high error scores in misreading the 10 B-Y plates. After evaluating the number of B-Y plates misread, we excluded from the test, the 3rd left plate '2', because this plate was often misread by those with normal colour vision.[14]

Subjective critical fusion frequency was measured with test apparatus II of Osaka University (Yagami Ltd, Japan). The frequency required to perceive a flickering light was determined three times to obtain the mean.

For VECP recordings, the electrode was placed at O_z and referred to the earlobe. The stimulus was a chequerboard pattern displayed on a black and white TV monitor. The pattern reversed at rates of 2, 4, 6, 8, 10, 12, 15, 20, and

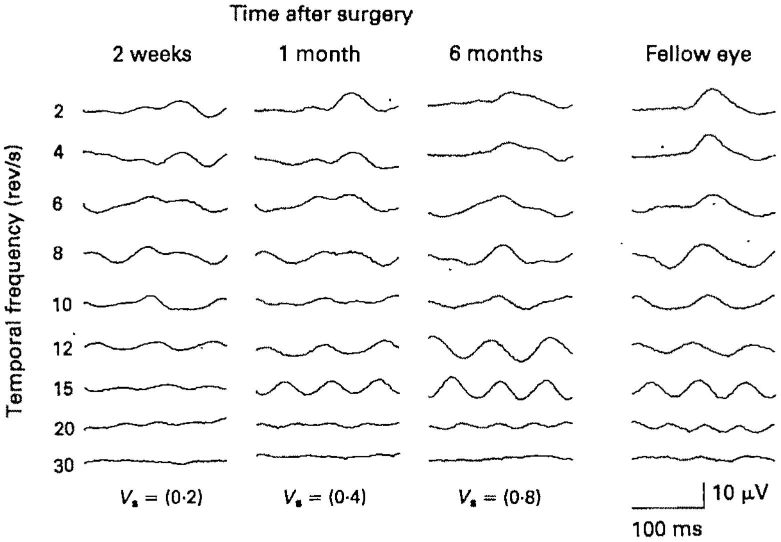

Figure 1 Actual pattern visual evoked cortical potential recordings after surgery in a 54-year-old female patient with rhegmatogenous retinal detachment involving the macula.

30 times per second. The chequer size and pattern field subtended 30′ and 7·8 deg by 10·5 degrees from a viewing distance of 180 cm. Contrast and mean luminance were kept at 80% and 41·5 cd/m^2, respectively. The patient fixed on a point in the centre of the pattern monocularly. The pupil was dilated, and an artificial pupil of 3 mm was used. One hundred responses were averaged (ATAC 350, Nihon Kohden Ltd, Tokyo), after passing the pre-amplifier (AVB9, Nihon Kohden Ltd, Tokyo) with a bandpass of 1·5 to 100 Hz, and printed by an X-Y recorder, in the positivity of the scalp electrode with an upward deflection (Fig 1). Peak latency of the first positive component (P_{100}) was measured in response to the transient stimulus of 2 rev/s.

The amplitude of the cortical potentials was measured between the top of the P_{100} component and the bottom of the following negative wave for the transient stimulus. For steady state potentials, the average of the peak-to-trough amplitude of sinusoidal waves was determined. Amplitudes were plotted as a function of temporal frequency on a log scale. The inverse U-shaped curve was obtained for each frequency range examined. At higher frequency ranges the amplitude decreased; a linear relationship existed between the amplitude and increasing log stimulus reversal frequency. The regression line was calculated, and VECP-critical fusion frequency (CFF) was determined by extrapolating the line to 0 μV amplitude[15] (Fig 2).

Results

Only visual acuity was measured preoperatively. Because the retinal detachment involved the macula the eyes were judged to be unable to provide reliable results in the other three test conditions because of poor visual acuity.

VISUAL ACUITY

The corrected visual acuity before and after operation is shown in Figure 3. It improved significantly up to 1 month after surgery, from a preoperative vision of mean 0·09 (SE 0·02) to 0·36 (SE 0·06). Thereafter a slight gradual improvement was observed for up to 2 months after surgery. No further recovery was observed thereafter, however, and visual acuity remained at 0·59 (SE 0·09) at 6 months. This level was significantly lower than that of the non-affected fellow eyes (1·10 (SE 0·07)) ($p<0\cdot01$).

Postoperative maximal visual acuity had no correlation with the duration of preoperative macular detachment (Fig 4).

COLOUR VISION

All of the non-affected fellow eyes of the patients passed both tests of colour vision. All of the affected eyes examined at 2 weeks after surgery passed the panel D-15 test. Nine of 14 eyes misread the B-Y plates of the pseudochromatic test at 2 weeks postoperatively, and underwent a repeated testing.

The mean number of those misreading the B-Y plates at five different times after surgery is shown in Figure 5. The number decreased significantly ($p<0\cdot05$) from 2 weeks to 2 months after surgery. Four eyes were still misreading the B-Y plates at 6 months after surgery.

SUBJECTIVE CRITICAL FUSION FREQUENCY (CFF)

Figure 6 showed the mean of CFF after surgery. At 2 weeks postoperatively the frequency was 33·6 (SE 0·9) Hz, which was significantly lower ($p<0\cdot01$) than that of the fellow eyes (41·7 (SE 0·6 Hz)). The frequency increased significantly at 1 month to 38·4 (SE 1·2) Hz ($p<0\cdot01$), but no further improvement was observed until 6 months postoperatively.

P_{100} PEAK LATENCY

The mean peak latency of the P_{100} component of the evoked potentials (Fig 7) that was obtained at 2 weeks after surgery was 137·5 (SE 3·1) ms was significantly prolonged ($p<0\cdot01$), when compared with that of the non-affected fellow eye (116·5 (SE 1·9) ms). The peak latency became shorter with time, but still was prolonged ($p<0\cdot01$) at 6 months after surgery.

VISUAL EVOKED CORTICAL POTENTIAL-CRITICAL FUSION FREQUENCY

We were able to test with certainty the VECP-CFF in eight of 14 eyes (Fig 8). Compared with

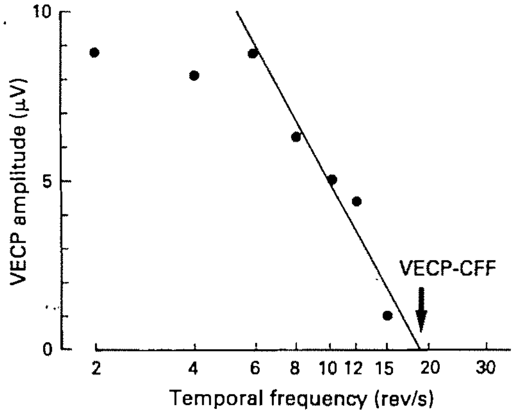

Figure 2 Visual evoked cortical potential (VECP) amplitudes as a function of log stimulus reversal frequency in a 59-year-old female patient. VECP-critical fusion frequency (VECP-CFF) was determined by extrapolating a regression line at a higher frequency range to 0 μV.

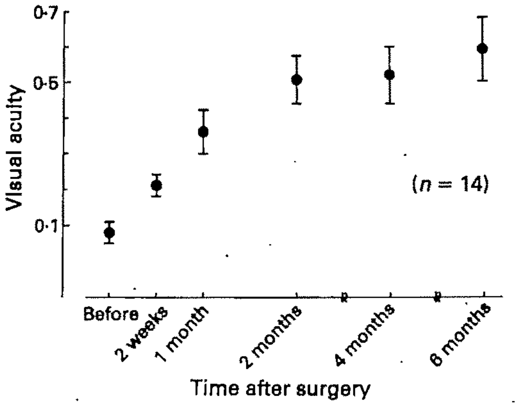

Figure 3 Mean (SE) visual acuity before and after surgery. The bars indicate ± standard errors (SE).

that of the fellow eye (28·1 (SE 1·7) rev/s), the mean value in the affected eye was significantly lower at 2 weeks (18·1 rev/s) (p<0·01). Thereafter, it began to increase gradually to 20·3 at 1 month, 22·7 at 2 months, 24·6 at 4 months, and 27·5 rev/s at 6 months. The increase from 2 weeks to that at 4 months was significant (p<0·01). No significant difference was observed in CFF after 2 months.

Discussion

After surgical repair ophthalmoscopically reattached retinas do not produce a satisfactory recovery of visual acuity, according to patients. One of the factors thought to influence visual recovery has been duration of preoperative macular detachment,[1–12] as reported precisely by Burton.[12]

The present study showed no such relation, although the number of cases is relatively low, because of the limited number of patients who could cooperate with electrophysiological measurements. Even the eye with a preoperative macular detachment of 6 weeks' duration improved to a visual acuity of 1·2. Only one eye, with a long-standing macular detachment of 6 months, demonstrated the worst visual acuity of 0·2.

Gundry and Davies[1] reported on one patient with a 1 day preoperative detachment. The visual acuity in that eye improved to 6/9, which was still lower than that of normal. The average maximal visual acuity after successful surgery showed improvements equal to half that of the level before detachment, which corresponded well with our results. Conversely, Chisholm and associates[4] reported that a visual acuity of 0·1 was the norm for the first postoperative year and 0·17 by the end of the second year.

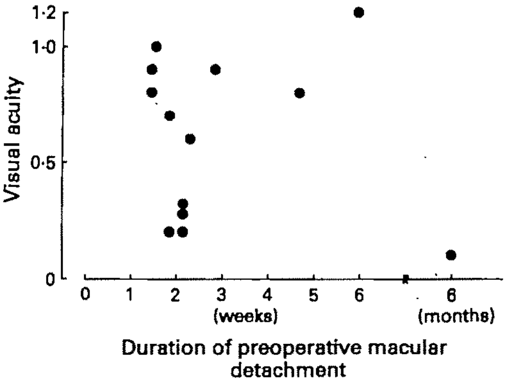

Figure 4 Maximal visual acuity after surgery plotted against a duration of preoperative macular detachment.

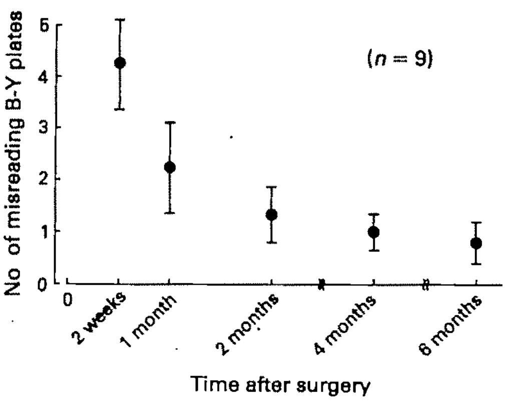

Figure 5 Mean number (SE) of misreading B-Y plates of the standard pseudochromatic test part II after surgery.

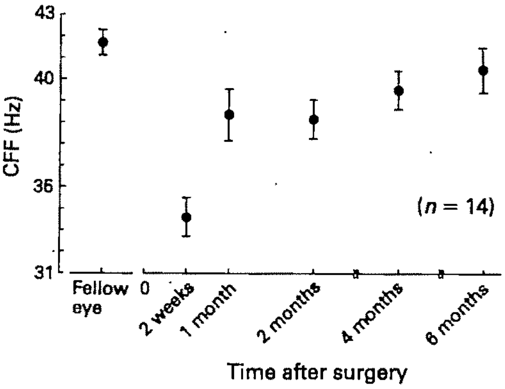

Figure 6 Mean (SE) subjective critical fusion frequency (CFF) after surgery.

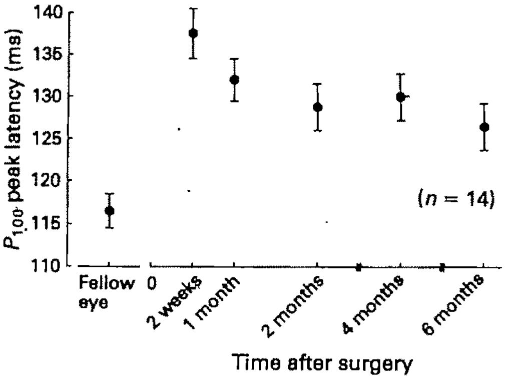

Figure 7 Mean (SE) peak latency of the P_{100} of pattern visual evoked cortical potentials after surgery.

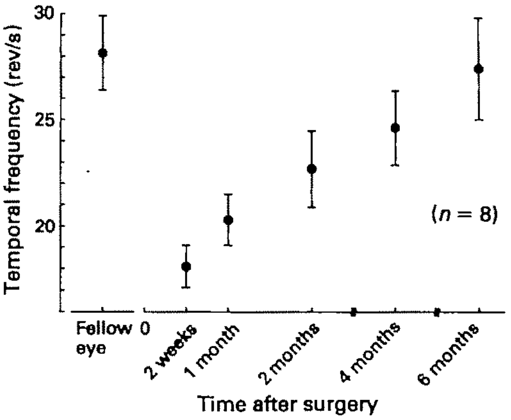

Figure 8 Mean (SE) pattern visual evoked cortical potential-critical fusion frequency (VECP-CFF) after surgery.

The time required for a steady maximum visual acuity was found to be within 2 months, as reported by Gundry and Davies,[1] Foulds *et al*,[2] Binder *et al*,[3] and Burton.[12]

Colour vision after surgery has shown B-Y defects, as based on the FM 100-hue test.[1-3] We also found B-Y defects with the use of the pseudochromatic test, which was sensitive for evaluating acquired colour vision deficiency.[14,16] Recovery of the B-Y defect was similar to that of visual acuity; B-Y defects recovered within 2 months to the preoperative level, with a residual defect. Foulds *et al*[2] described a residual defect of hue discrimination that lasted for 2 years in those eyes which were followed up.

To our knowledge no report has tested both subjective and objective critical fusion frequency in follow-up studies of retinal detachment. In the present study, we showed less decrease and faster recovery of subjective critical fusion frequency in comparison with visual acuity and colour vision, although the frequency reflects visual function from the ganglion cell layer of the retina to the visual cortex.

To test central visual functions objectively, either focal macular electroretinography or visual evoked cortical potentials can be used at present. A focal electroretinogram requires a fine recording set-up to avoid the effects of stray light. On the other hand, pattern visual evoked cortical potentials are gradually becoming popular in clinical use to examine central vision from the macular region to the visual cortex.

Using pattern evoked cortical potentials, we found that the prolongation of the P_{100} peak latency after surgery shortened with the improvement of visual acuity and colour vision, and that the evoked potentials-critical fusion frequency based on the amplitude increased with recovery of visual acuity. We have to be aware of the importance of pattern evoked cortical potentials as a central visual function test.

With regard to the pathological features of the reattached retina, several series of experiments have been described by Machemer's group.[17-19] Machemer,[17] and Kroll and Machemer[18] experimentally induced retinal detachment in the owl monkey and observed the anatomical findings in the reattached retina after surgery. They demonstrated that the retina became normal within 8 weeks after regeneration and reposition of receptor cell outer segments. In another study,[19] the authors used the rhesus monkey to show the rod repositioned within 1 month, which was an earlier response compared with the cone. Our results, based on both subjective and objective measurements, suggested lesser functional recovery of receptor cells at the central retina.

Sarin and McDonald[20] found optic atrophy secondary to vascular occlusion after reattachment procedures. As far as our pattern evoked potential results are concerned minor pathological changes of the optic nerve were suggested although none was observed ophthalmoscopically.

Reduced functional recovery after successful surgery might be due not only to the anatomical changes but also to biochemical disorders, such as decreased neurotransmitters in the visual pathway. Delay of pattern evoked potentials has been found in parkinsonian patients who lack dopamine[21] and in elderly subjects who have reduced levels of dopamine.[22] The species difference between the monkey and human might explain the discrepant findings.

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Fibronectin synthesis in subretinal membranes of proliferative vitreoretinopathy

P Hiscott, H A Waller, I Grierson, M G Butler, D L Scott, Z Gregor, I Morino

Institute of Ophthalmology, Department of Pathology
P Hiscott

Department of Clinical Science
P Hiscott
I Grierson

Departments of Immunology and Rheumatology, St Bartholomew's Hospital
H A Waller
M G Butler
D L Scott

The Vitreoretinal Unit, Moorfields Eye Hospital
P Hiscott
Z Gregor

Department of Ophthalmology, Kobe University, Japan
I Morino

Correspondence to:
Dr P Hiscott, Institute of Ophthalmology, 17-25 Cayton Street, London EC1V 9AT.

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Abstract

In situ hybridisation and immunohistochemical studies were conducted on six surgically excised subretinal membranes of proliferative vitreoretinopathy to investigate whether displacement of retinal pigment epithelial and glial cells to subretinal membranes was associated with fibronectin production by the subretinal membrane cells. Fibronectin messenger RNA (mRNA) and fibronectin immunoreactivity were observed in some cells in all of the subretinal membranes studied and up to 30% of the cells in individual specimens showed intense labelling for fibronectin mRNA. The results support the concept that the cells in subretinal membranes produce fibronectin. Locally produced fibronectin may play a role in subretinal membrane cohesion, and displacement of retinal pigment epithelial and glial cells from their normal location may induce the cells to manufacture fibronectin. Fibronectin production may be more prominent in migrating subretinal cells.

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Proliferative vitreoretinopathy (PVR) is a complication of retinal detachment and is characterised by the development of contractile, scar-like fibrocellular membranes on either side of the detached neuroretina (epiretinal and subretinal membranes).[1] The contractile epiretinal and subretinal membranes of PVR have a similar cellular composition,[2-4] although contractile subretinal membranes appear to have a larger retinal pigment epithelial component and smaller glial component than epiretinal membranes,[3 5 6] and the extracellular matrix of PVR membranes at both sites contains collagens and glycoproteins, including fibronectin.[7-10] Fibronectin has a number of important biological functions such as a role in cell-cell and cell-substratum adhesion,[11-13] and the adhesive properties of fibronectin may contribute to the cell-cell and cell-extracellular matrix cohesion in early PVR membranes.

In PVR, epiretinal glial and retinal pigment epithelial cells are replete with fibronectin messenger RNA (mRNA) whereas cells in the subjacent detached neuroretina and retinal pigment epithelial monolayer exhibit little or no fibronectin message, suggesting that the ectopic retinal pigment epithelial and glial cells produce abundant glycoprotein while those in situ in the retina do not.[14] The 'up regulation' of fibronectin production by the displaced cells probably contributes to the glycoprotein in epiretinal membranes but the mechanism for the change in gene expression for fibronectin is obscure.

One possible explanation for the activation of fibronectin production in the ectopic cells is that displacement of the retinal pigment epithelial and glial cells from their normal location stimulates, or removes inhibitions from, the cells to produce fibronectin, in which case fibronectin should be produced by the cells supplanted to sites other than the vitreoretinal junction. One such location is within the contractile subretinal membranes of PVR where retinal pigment epithelial and glial cells are displaced into the developing fibrocellular tissue.[2-6] Therefore, to investigate further the hypothesis that displacement of pigment epithelial and glial retinal cells causes them to produce fibronectin, we examined the cells in PVR subretinal membranes for the presence of fibronectin mRNA employing an in situ hybridisation technique. The results were correlated with the distribution of fibronectin immunoreactivity in the specimens.

Materials and methods

CONTROL TISSUE

Formalin fixed, paraffin embedded synovial tissues, from patients with rheumatoid arthritis known to contain abundant fibronectin mRNA and show strong fibronectin immunoreactivity,[15] were used as positive controls for the hybridisation experiments and immunohistochemical procedures.

SUBRETINAL MEMBRANES

Six subretinal membranes removed during pars plana vitrectomy for PVR were used in the study.

PREPARATION OF SINGLE-STRANDED RNA PROBES

A cDNA clone (pFH1) representing about one quarter of the estimated size of human cellular fibronectin mRNA[16 17] was provided by Dr F E Baralle, Sir William Dunn School of Pathology, University of Oxford and subcloned into pGEM blue (Promega, WI, USA) to produce single-stranded RNA anti-sense and sense (control) probes, as previously described.[14 15]

Transcription was conducted incorporating digoxigenin-11-UTP (Boehringer Mannheim, GmbH) with the standard transcription reaction using SP6 or T7 RNA polymerase (Boehringer). To achieve optimal probe length for tissue penetration alkaline hydrolysis was carried out to give an average probe length of 100 bases.[18] To prevent digoxigenin loss into the organic phase phenol/chloroform extraction was omitted during probe purification.[19] To ensure the correct size of transcripts and the success of hydrolysis the probes were checked by 2% denaturing agarose gel electrophoresis and northern blotting.[20] Nitrocellulose filters were blocked with

3% normal sheep serum in phosphate buffered saline (PBS; pH 7·4) and subsequent immunochemical detection of probes was carried out using sheep anti-digoxigenin alkaline phosphatase conjugate (Boehringer) at 1:5000 in 3% normal sheep serum/PBS. Development was with NBT/BCIP (Sigma, Poole, UK) in TRIS buffered saline, pH 9·5 (0·3 mg/ml NBT; 0·2 mg/ml BCIP; 0·1 M TRIS; 0·1 M sodium chloride; 0·005 M magnesium chloride). Probes were stored at −20°C until used.

HYBRIDISATION HISTOCHEMISTRY

Subretinal membranes were fixed for between 4 and 48 h in 10% formol-saline, dehydrated in graded concentrations of ethanol and embedded in paraffin wax.

Sections (6 μm thick) of wax-embedded tissue were cut, dewaxed, permeabilised, treated with proteinase K, post-fixed, dehydrated in methanol and air-dried.[14] The anti-sense (SP6, fibronectin transcript) probe was then applied at a concentration of 1:100 (approximately 0·1 μg/ml) in hybridisation medium and the preparations were hybridised overnight under coverslips at 37°C in a humid chamber. During all the procedures up to and including hybridisation, RNAse free conditions were employed.[20] The coverslips and unbound probe were removed as previously described.[14]

Sites of probe binding were visualised immunohistochemically using the antibody to digoxigenin. Non-specific antibody binding was blocked by a 20 minute incubation with 3% normal sheep serum/PBS, sheep anti-digoxigenin antibody-alkaline phosphatase conjugate was applied to the sections for 30 minutes at a concentration of 1:200 in 3% normal sheep serum/PBS. The sections were washed in PBS and the reaction sites visualised blue/black in colour using NBT/BCIP (as described above) overnight. The preparations were washed, counterstained with haematoxylin and mounted in glycerol.

Procedural controls included (1) pre-treatment of sections with 20 μg/ml RNAse in TRIS/EDTA buffer (0·5 M sodium chloride, 10 mM TRIS hydrochloride pH 8·0, 1 mM EDTA) for 30 minutes at 37°C to remove all tissue RNA, and (2) incubation of sections with the sense (T7) probe at a concentration of 1:100 instead of the anti-sense probe.

FIBRONECTIN IMMUNOHISTOCHEMISTRY

Serial sections (6 μm thick) to those used for in situ hybridisation were immunostained for fibronectin with an antiserum raised in rabbit to human plasma fibronectin (Dako) or a monoclonal antibody from Sigma (which gave similar staining patterns). The immunostaining was conducted as previously described[7] and the immunoperoxidase technique was used throughout this investigation, employing 3-amino-9-ethylcarbazole as chromogen. Some sections were counterstained with Mayer's haematoxylin. Other sections were stained with haematoxylin and eosin. Controls were applied to the immunostaining procedures as previously described.[7] In addition, where sufficient tissue was available in the specimens the retinal origin of the subretinal cells was confirmed by immunotyping their intermediate filaments (using glial fibrillary acidic protein to detect glial cells and cytokeratin to demonstrate retinal pigment epithelial cells).[21 22]

The hybridisation and immunohistochemical preparations were examined and compared by bright field and differential interference contrast microscopy. Semi-quantitative estimates of hybridisation and immunohistochemical labelling were made by comparing the 'test' sections with controls. Labelling equivalent to that seen in negative controls (for example, sense probe treated sections or immunohistochemical negative control sections) was termed 'background labelling', while positive labelling at a level equivalent to the strongest seen in positive rheumatoid synovium was designated 'intense

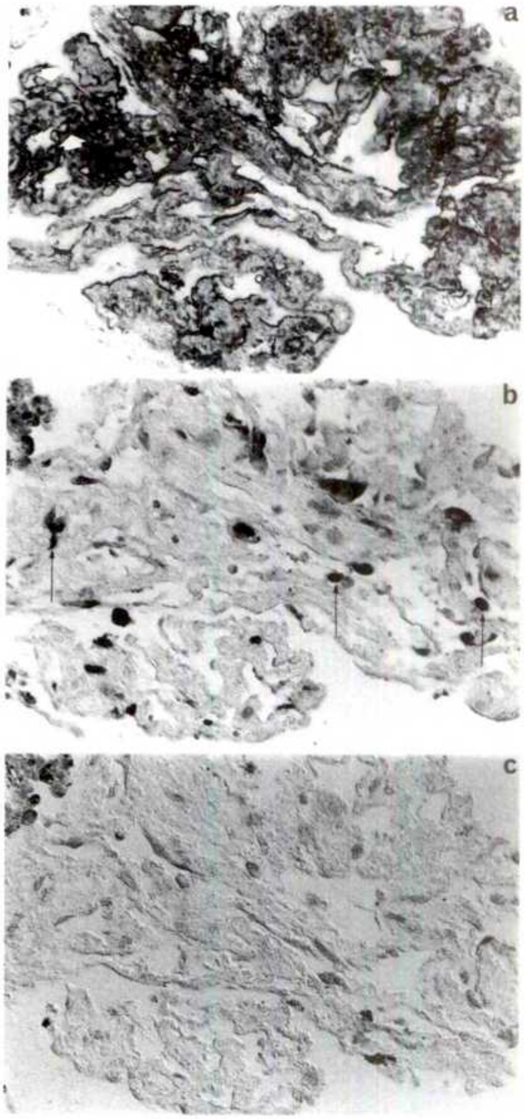

Figure 1 *Serial sections from a subretinal membrane seen by differential interference contrast microscopy. (a) Stained with the immunoperoxidase technique for fibronectin (no counterstain). Much of the membrane shows intense fibronectin immunoreactivity with a distinct fibrillar pattern (white arrows). (b) Labelled with the anti-sense probe for fibronectin mRNA and counterstained with haematoxylin. Many of the cells in the section show intense labelling for fibronectin mRNA (black arrows). (c) Labelled with the sense (control) probe. No labelling is seen. A group of pigmented cells is seen in the top left corner. All ×230.*

labelling', Labelling between background and intense levels was called 'moderate labelling'.

Results

CONTROLS

Sections of rheumatoid synovium hybridised with the anti-sense probe showed moderate or intense labelling in the synovial lining cells whereas sections hybridised with the sense probe or pretreated with RNAse exhibited only background labelling. The synovium showed moderate or intense immunoreactivity for fibronectin while no immunolabelling occurred in the negative procedural controls. Subretinal membrane sections labelled with the sense probe showed only background labelling levels (Fig 1).

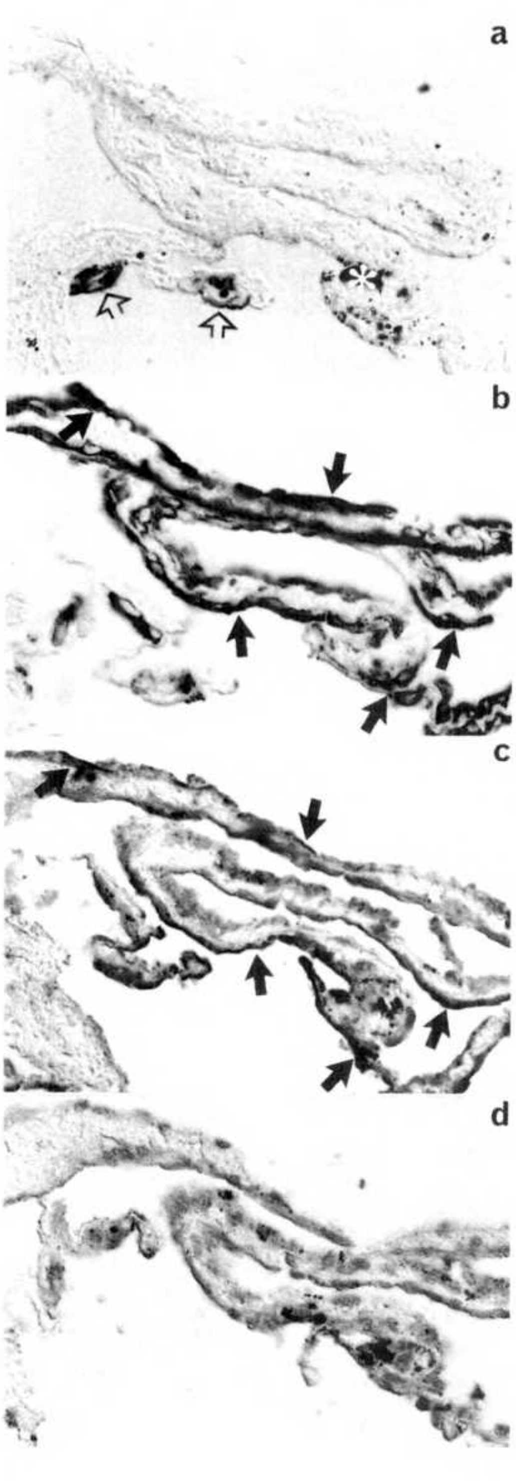

Figure 2 Differential interference contrast micrographs of sections from a subretinal membrane. (a) Stained by the immunoperoxidase technique (no counterstain) for glial fibrillary acidic protein reveals a small glial component in the membrane (open arrows). Pigment is present in the tissue (asterisk). (b) Stained by the immunoperoxidase technique (no counterstain) for cytokeratins shows prominent retinal pigment epithelial layers (arrows). (c) Labelled with the anti-sense probe for fibronectin mRNA and counterstained with haematoxylin. Many of the pigment epithelial cells show moderate or intense labelling for fibronectin message (arrows). (d) Labelled with the sence (control) probe and counterstained with haemotoxylin. No labelling is seen. All ×290.

Table 1 Fibronectin mRNA in the cells of PVR subretinal membranes

Subretainal membrane no	*Proportion of cells showing moderate labelling for fibronectin mRNA (%)*	*Proportion of cells showing intense labelling for fibronectin mRNA (%)*
1	20	0
2	20	30
3	10	10
4	50	0
5	30	20
6	20	10

CELLS IN SUBRETINAL MEMBRANES

All six subretinal membranes contained cells which labelled with the anti-sense probe (Figs 1 and 2). There was some variation between the membrane specimens in the numbers of cells which contained fibronectin mRNA. Between 20% to 50% of cells showed levels of labelling above background with the anti-sense probe (Table 1). In four membranes between 10% to 30% of the total cells in the specimen showed intense labelling with the probe, the remaining positive cells labelling at moderate levels. Labelling was seen in both isolated cells and cells aggregated in layers in the subretinal tissue, including cells in layers at apparent specimen surfaces (Fig 2). Moderate to strong fibronectin immunoreactivity was also observed in all the subretinal membranes (Fig 1). Enough tissue was present in the specimens to confirm that some of the cells which contained fibronectin mRNA were retinal pigment epithelial (four membranes; Fig 2) or glial (one membrane) in origin. It was not possible to detect a quantitative difference between cell types in the intensity of labelling for fibronectin mRNA.

Discussion

Our results confirm that fibronectin contributes to the extracellular matrix in the contractile subretinal membranes of PVR, demonstrate that many subretinal cells contain mRNA for the glycoprotein, and affirm that cells in subretinal membranes produce fibronectin. Moreover, the results are in keeping with the observation that fibronectin is synthesised by retinal pigment epithelium in vitro,[23 24] since retinal pigment epithelial cells are a major component of subretinal membranes.[1-6]

The production of fibronectin by cells in subretinal membranes represents a marked behavioural change from the activities seen in the cells in situ in attached and even detached adult human retina, since retinal pigment epithelial and glial cells in situ lack appreciable fibronectin immunoreactivity and mRNA.[14] This 'up regulation' of fibronectin production by retinal pigment epithelial and glial cells in subretinal membranes may be caused by one of several mechanisms. One possibility is that inflammatory mediators within the vitreous and subretinal fluids of eyes developing PVR subretinal membranes, or factors derived from inflammatory cells in evolving membranes, may stimulate fibronectin production. It has been shown that hepatocytes manufacture fibronectin in the presence of interleukin-6.[25] However, the apparent absence of fibronectin production in the adjacent neuroretina and retinal pigment epithelial monolayer is counter to this theory. Alternatively, the observation of fibronectin synthesis in the subretinal, as well as the epiretinal,[14] component of PVR is in keeping with the theory that it is the displacement of retinal pigment epithelial and glial cells from their normal location which induces, or removes constraints from, the cells to produce fibronectin.

Some of the fibronectin in subretinal membranes may also be derived from plasma fibronectin, since fibronectin enters the vitreous and subretinal space following breakdown of the blood retina barrier in retinal detachment and PVR.[26–28] The developing subretinal 'scars' may then incorporate the plasma-derived fibronectin. Indeed, moderate to strong fibronectin immunoreactivity was observed in all the subretinal membranes studied irrespective of the proportion of cells expressing fibronectin mRNA. However, much of the fibronectin content of the membranes is likely to emanate from the subretinal cells themselves since cell surface fibronectin is thought to be insoluble[13] and therefore is likely to accumulate in the tissue. Thus both plasma and locally-produced fibronectin probably contribute to the tissue.

The accumulation of both plasma and locally-produced fibronectin has been described in reparative processes elsewhere in the body[13, 29] and in this respect subretinal membrane formation may be likened to an evolving scar. However, in other respects subretinal membranes differ from healing wounds – for example, the absence of a prominent vascular component,[6] and it remains to be seen whether the fibronectin is as important in subretinal membrane formation as it is wound healing in general.

The production of fibronectin by subretinal membrane cells does not imply that fibronectin is important in the pathobiology of the tissue per se. However, the abundance of message in some subretinal membranes and the recent demonstration of fibronectin receptors on cells displaced into PVR epiretinal membranes[30] is in keeping with the concept that the glycoprotein plays a role in subretinal membrane development.

In early PVR, plasma-derived fibronectin is likely to be involved in chemotactic recruitment of cells into the developing subretinal tissue. As the subretinal membrane cells themselves begin to produce fibronectin, the insoluble cell surface glycoprotein may contribute to the cohesion of the tissue and potentiate the chemotactic stimulus in the membrane. Indeed, work in our laboratory suggests that retinal pigment epithelial cells may be migrating into already well established subretinal membranes.[31, 32]

Migrating cells may not only be attracted into developing subretinal membranes by fibronectin but could specifically be producing the glycoprotein since migratory cells in other pathological tissues can produce fibronectin.[33] Interestingly, fibronectin mRNA was particularly noted in cells at apparent surfaces of the subretinal membranes where migratory cells may be expected. If migrating cells produce fibronectin in evolving membranes, it is possible that a single agent could prevent both recruitment of cells into and cohesion within developing membranes and provide a new avenue of therapeutic intervention in the process.

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Prenatal exclusion of Norrie's disease

R M Redmond, C A Graham, E D Kelly, M Coleman, N C Nevin

Abstract

We report on the use of DNA marker probes and linkage analysis to exclude Norrie's disease in the male fetus of a high risk carrier. There are no clinical markers in females carrying the Norrie's disease gene; thus DNA linkage analysis is an essential technique in the management of families 'at-risk' for this severe ophthalmic disease. The principles of DNA linkage are discussed.

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The Queen's University, Belfast, Department of Ophthalmology
R M Redmond

Department of Medical Genetics
C A Graham
E D Kelly
N C Nevin

Institute of Molecular Medicine, University of Oxford
M Coleman

Correspondence to:
Mr R M Redmond, Moorfields Eye Hospital, City Road, London EC1V 2PD.

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Norrie's disease (ND, McKusick catalogue number 31060) is an X-linked recessive form of blindness. The main features of ND, as described by Warburg,[1] are:

(1) bilateral retinal dysplasia and detachment evident at birth or in early infancy, with subsequent cataract formation, opacification of the cornea, and optic atrophy. Chronic hypotony is usual, following a period of secondary glaucoma and ocular pain;

(2) progressive mental deterioration is common, with onset usually in early childhood and psychotic behavioural patterns developing later;

(3) variable hearing loss.

Other features include growth retardation and wasting, a narrow nasal bridge, large ears, hypogonadism, or undescended testes.[2]

The dense white or yellow-white retrolental mass due to detached, dysplastic retina results in leukocoria and requires urgent clinical investigation and possible enucleation in order to exclude retinoblastoma (see Fig 1). Eventual phthisis bulbi has led to ND being mis-diagnosed as congenital microphthalmia.[3] No biochemical, ocular, or systemic abnormality has been reported in carrier females.

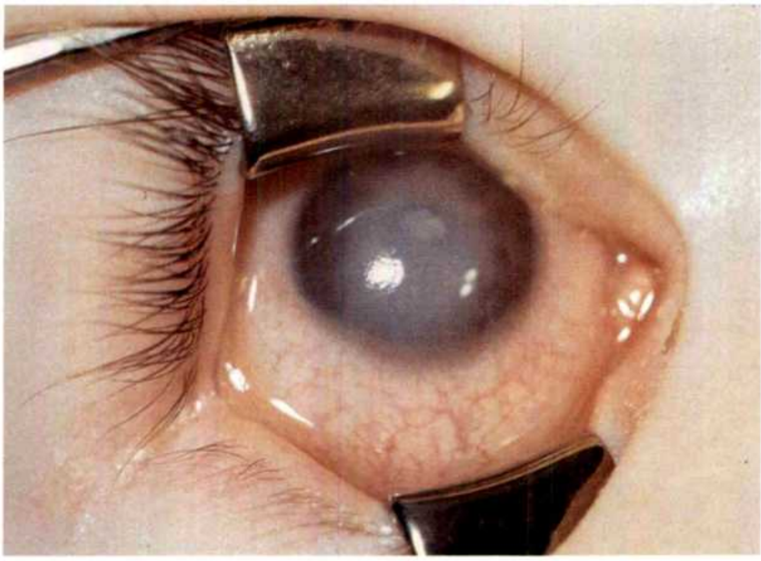


Figure 1 Right eye of proband II6 showing central corneal oedema, shallow anterior chamber, atrophic iris, miosed pupil, and cataract.

Pathology

The primary pathological process in ND is an abnormal retinal embryogenesis and retinal dysplasia.[4] Thus the primary tissue defect is neuroectodermal in origin. Histological examination reveals a dysplastic retina with rosettes of poorly differentiated photoreceptors. The rosettes are encased in cords of proliferating embryonic cuboidal epithelium. Secondary changes comprise neuroglial proliferation from the retina into the early vitreous, retinal detachment, and haemorrhage. With fibrous contraction a retrolental mass forms in the hypotonous, microphthalmic eye.

Linkage studies

Gene loci are linked if they are physically close on a chromosome. Their separation by meiotic 'crossing-over' (recombination) is therefore of low probability. A single cross-over event between two homologous chromosomes is illustrated in Figure 2. Linkage analysis of a human pedigree uses observed recombination events between the disease gene and marker loci to calculate the proportion of recombinant individuals (the recombination fraction) and LOD score for the disease gene and a chosen marker. An LOD score is the Logarithm (base_{10}) of an ODds ratio:

$$\frac{\text{odds the two loci are linked with recombinant fraction } \theta}{\text{odds the two loci are unlinked at recombination fraction } 0{\cdot}5}$$

From the calculated 'genetic' distances between the disease gene and the accurately located markers, a map can be constructed giving the most probable location of the disease gene. Conversely, the most tightly linked probe markers can be used to track the gene through the generations of a human pedigree. Two DNA probe markers used in this study are radioactively-labelled short lengths of single-stranded DNA (oligonucleotides) which will hybridise to unique nucleotide sequences in single-stranded X chromosome DNA. The creation of restriction fragment length polymorphisms (RFLPs) and their identification with such DNA probes is shown in Figure 3.

A combination of DNA linkage studies and deletion mapping has assigned the ND locus to Xp11·3, a region near the centromere on the short arm of the X chromosome. In 1985, Gal *et al*[5] and Bleeker-Wagemakers *et al*[6] detected close linkage between ND and locus DXS7 (DNA marker L1·28). Recombination between these two loci was reported by Katayama *et al*[7] and Ngo *et al*[8] and a maximum LOD score of 7·46 at 1% recombination implied that the ND

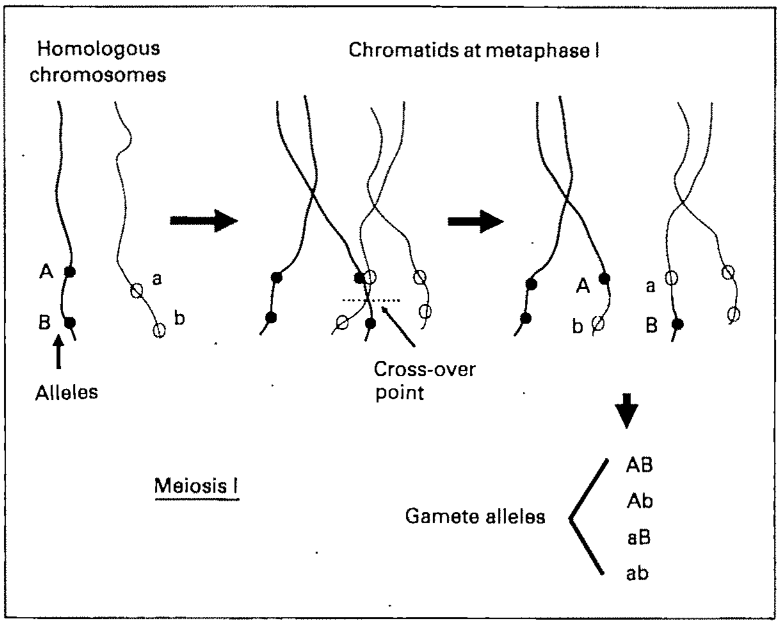


Figure 2 Diagram of a single cross-over between the arms of paired homologous chromatids in metaphase of meiosis I. An average of 55 cross-over events occur in male meiosis; more in the female.

gene locus may be approximately 1 million nucleotide base pairs (1Mb) away from the DXS7/L1·28 marker site.

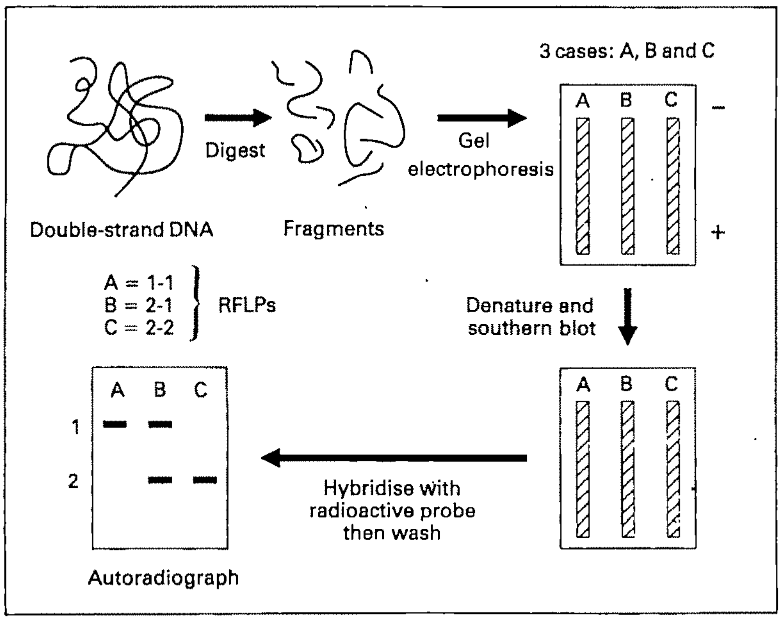
Further DNA markers for ND are listed in Figure 4; they are all located on the proximal short arm of the X chromosome. Recent accounts of relevant molecular genetic techniques in the ophthalmic literature have been provided by Jay and Inglehearn,[9] Esakowitz *et al*,[10] and by Redmond *et al.*[11]

Material

The Northern Ireland family in this report was originally described by Johnston *et al.*[12] There are three affected males as shown in the partial pedigree (Fig 5).

The proband (II6) was born in 1970. In the first month of life he was noted to have right microphthalmos with a hazy cornea, a shallow anterior chamber, and elongated ciliary processes. A yellow-white retrolental mass and ectropion uveae developed. The initial diagnosis was persistent hyperplastic primary vitreous, but by the age of 1 year he had developed bilateral corneal oedema with miosed pupils, atrophic, vascularised irides, and cataracts. Mental retardation was noted.

Figure 3 Diagram illustrating the process of DNA digestion with restriction enzymes and subsequent electrophoresis to separate the polymorphic fragments, Southern blotting and scoring of the RFLPs.



The proband's cousin (II9) was traced by Johnston *et al.*[9] This boy's left microphthalmic eye had been previously enucleated as retinoblastoma could not be excluded. Histological examination of the globe confirmed the diagnosis of ND.

The possible ND carrier II2 became pregnant and required urgent genetic counselling and prenatal investigation with fetal sexing and DNA linkage analysis of amniotic fluid cells.

Methods

DNA from 13 members of the family was extracted from venous blood leucocytes by the method of Jeanpierre[13] and the sample concentrations were standardised to approximately 500 μg/ml. The restriction enzymes *TaqI* and *EcoRI* were used to digest DNA aliquots and the fragments were separated by electrophoresis in 0·8% agarose gels and transferred to Hybond N nylon membranes (Amersham) by vacuum blotting.

The *TaqI* 'blot' was hybridised with the probe L1·28. The RFLPs identified by *TaqI*/L1·28 comprise a 2-allele system, the fragments (alleles 1 and 2) being 12 and 9 kilobases in size with general population frequencies of 68% and 32% respecitvely. The *EcoRI* 'blot' was hybridised with the multi-allelic probe. M27β. With this probe females have a 95% chance of being heterozygous. Males, with only one X chromosome, are hemizygous and show only one allele.

The polymerase chain reaction (PCR) was used with primers for the DXS426 locus.[14] This

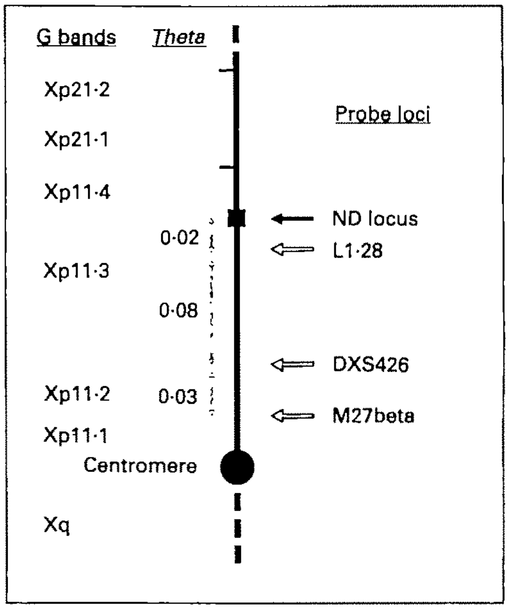
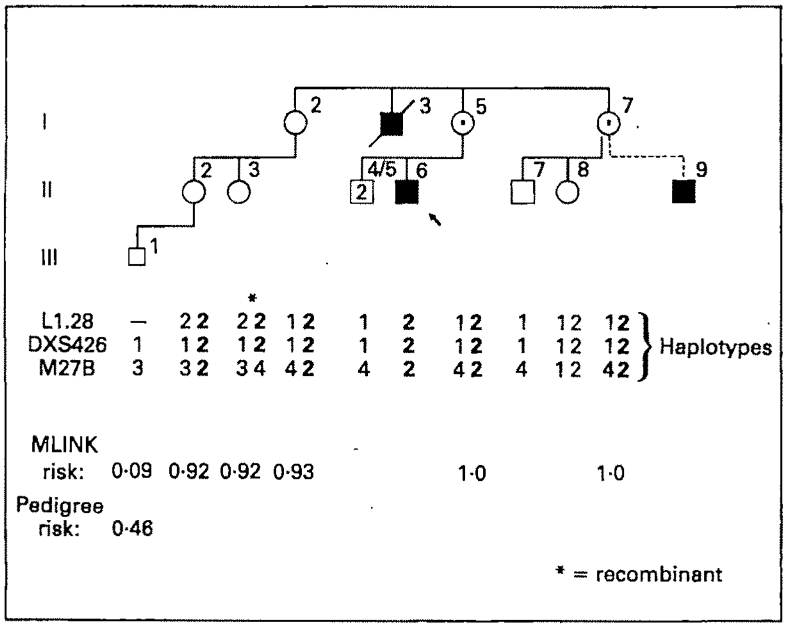


Figure 4 Schematic diagram of the proximal short arm of the X chromosome (Xp). The Norrie disease gene is represented by a solid block and the relative location order of DNA markers (linked to ND) is shown. Recombination values (Theta) *represent genetic 'distances' between the loci measured in centiMorgans (1 cM=1% recombination=1 Mb).*


I
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M27B 3 3 2 3 4 4 2 4 2 4 2 4 1 2 4 2
Haplotypes
MLINK
risk: 0·09 0·92 0·92 0·93 1·0 1·0
Pedigree
risk: 0·46
* = recombinant

Figure 5 Partial pedigree: DNA linkage analysis results for individual III1 are prenatal, and predicted the high likelihood of an unaffected male being born. Affected males are shown as solid squares; I3 is deceased. Females I5 and 7 are marked as obligate carriers as they have affected sons.

locus is a stretch of DNA in which cytosine/adenine dinucleotides are repeated a variable number of times and the locus is thus polymorphic. The repeated cycles of the reaction 'amplify' the polymorphic chains of DNA to a concentration far greater than that of irrelevant DNA. After gel electrophoresis, radioactive labelling is unnecessary as the amplified polymorphic bands fluoresce when the gel is stained with ethidium bromide under UV light.

The results for the three polymorphisms are shown in Figure 5, with the disease associated haplotype shown in bold type. The computer program MLINK v5.03[5] has been used to calculate disease and carrier status risks for key individuals using the recombination values given in Figure 4.

Discussion

The affected male II6 from whom DNA analysis is available, has the haplotype [2,2,2] for the three markers used. No unaffected male shows this haplotype and it is likely that the disease gene is linked to this haplotype. The sisters, I5 and I7, both obligate carriers for the ND gene, share the same genotype. Carrier risk estimation calculated by MLINK v5.03 for the female I2 is 93·4%.

The daughters of I2 (II2 and II3) have had DNA analysis using the same markers. II2 has inherited the [2,2,2] haplotype from her mother, whereas II3 has inherited [2,2,4], showing a crossover with the marker farthest from the ND locus (M27β). This does not alter the carrier risk estimation which was computed as 91·5% for both daughters. II2, at high risk of being a carrier, became pregnant. An early amniocentesis[16] was carried out at 10 weeks' gestation. Fluorescence Y chromatin analysis revealed that the fetus was male. Amniocytes were cultured for 2 weeks and DNA was extracted for prenatal diagnosis using the multi-allelic markers M27β and DXS426.

The fetal haplotype was [-,1,3] for these markers (see Fig 5). Thus it was likely that III1 had not inherited the ND gene. The risk of being affected was computed as 9% with MLINK v. 5.03. By comparison, risk estimation based on the pedigree alone indicates a 46% likelihood of an affected child (III1). The parents were advised of the risk estimation and the pregnancy continued to term. A healthy male baby was delivered.

Linkage analysis using multi-allelic probes has a high probability of being informative in individual families, and provides more accurate genetic counselling. The application of molecular biology techniques, as demonstrated by this case, will help to reduce the incidence of severe inherited ophthalmic disease.

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MINI REVIEW

Low tension glaucoma – its place in modern glaucoma practice

One of the commonest referrals to a glaucoma unit for advice on further management is the patient with 'progressing low tension glaucoma'.[1] There is often considerable disquiet not only about the correctness of the diagnosis but also about what, if any, treatment is appropriate. In this review current ideas on the diagnosis, prevalence, and characteristics of low tension glaucoma are set out, and some of the many limitations to our knowledge noted, along with possible treatments.

Definition

Low tension glaucoma may be defined as a variety of primary open angle glaucoma, having characteristic glaucomatous cupping and field loss, an open angle, and an intraocular pressure (IOP) within the normal range, with no other cause for these changes. Strictly speaking as the IOP is 'normal' rather than 'low' it should be called normal tension glaucoma. Common usage will ensure that the older title will continue for a while yet.

When diagnosing low tension glaucoma it is necessary to remember how difficult it is to define 'normal' pressure. Mean IOP has been derived from studies of large or 'total' Caucasian adult populations. Two standard deviations above this mean would include 96% of the population and include most of the normals. For the populations studied this 'upper limit of normal' lies near 21 mm Hg. Glaucomatous disease developing in an eye with IOP at or below this level can be said to have 'normal tension glaucoma'. The arbitrariness of this definition will be seen when it is remembered that the IOP in Caucasians rises with age in line with the systolic blood pressure,[2] yet the 21 mm Hg level is taken as the average for all adults; for a young adult Caucasian this level may therefore be too high, and conversely for an octagenarian it may be too low. The problem is further confounded on translating the results for Caucasians to other populations. For example Japanese adults show a fall in IOP with age,[3] again in line with systemic blood pressure. The mean IOP for an elderly Japanese patient is lower than for his Western counterpart, and so is his upper limit of normal. There will be a far greater prevalence of low tension glaucoma in Japan if definitions of normal based on Western pressure levels rather than Japanese ones are used, and this is the case. Of greater relevance to the urban populations in the United Kingdom is the fact that while West Africans have low IOPs (with large cup/disc ratios) with large numbers of 'low tension glaucomas' some of their Caribbean descendants may not, having aquired the IOP and systolic blood pressure consequences of a Western diet.

Eyes being considered for the diagnosis of low tension glaucoma should have diurnal curve measurements to rule out pressure spikes. A past history of trauma, treatment with topical steroids, or inflammation could all point to episodes of raised intraocular tension. The nerve fibre layer type of visual field defect could originate from a lesion behind the eye. All contenders for the diagnosis 'low tension glaucoma' should be investigated with these thoughts in mind, and the diagnosis should not be considered until episodic ocular hypertension and other causes of optic atrophy have been excluded. It should be noted in passing that it can be very difficult, on the basis of a single examination, to rule out anterior ischaemic optic neuropathy as the cause of the visual loss, and a final diagnosis may have to await evidence of progression of the visual field defect.

It can be seen that the inclusion of a patient in the category 'normal tension glaucoma' should be based on local rules and that there is no sharp dividing line between normal tension glaucoma and its more common counterpart 'high tension glaucoma'. For a Caucasian patient however the presence of glaucomatous field loss and cupping in an eye with open angles and an IOP *consistently* below 22 mm Hg suggests the diagnosis of normal tension glaucoma, which would be confirmed upon progression of the visual field defect.

Prevalence

Glaucoma screening of total populations reveals a surprisingly high figure for normal tension glaucoma, figures of up to 35% of all open angle glaucomas have been reported.[4] On rescreening these eyes the IOP is often found to be above normal so the figure for normal tension glaucoma in the community falls. A truer figure for Caucasians is probably nearer 15% of all primary open angle glaucoma patients.

Characteristics

A large number of studies have been carried out comparing the features of eyes with normal tension glaucoma as defined above with eyes having high tension glaucoma. There have been no detected differences in the topography of the optic disc[5 6] but the incidence of splinter haemorrhages on the optic disc is higher in normal than in high tension glaucoma.[7 8]

Comparisons of the visual field have tended to agree that while the visual field defects in normal tension glaucoma may be more likely to show dense defects close to fixation, this is not diagnostic.[9-14] It has been suggested that the uninvolved hemifield is more likely to be 'normal' without evidence of diffuse visual loss in normal tension glaucoma.[15 16] This finding has not been confirmed by other studies and seems to have been due to the analysis method used. The nature of the visual field loss would appear the same in normal tension and high tension glaucoma.

Though there is little difference in male:female ratios in patients with high tension glaucoma, the ratio in normal tension glaucoma is 1:2. There are no apparent rheological, haematological, or vascular differences between patients with normal and high tension glaucoma.[17 18]

Subgroups of normal tension glaucoma

Greve and his group in Amsterdam have proposed that normal tension glaucoma be divided into different subgroups based on differing clinical characteristics. In so doing they have emphasised the association of normal tension glaucoma with myopia, focal ischaemic episodes, and 'burnt out glaucoma' – senile sclerotic glaucoma.[19] While this descriptive classification highlights different possibilities in pathogenesis of the disease, it remains to be seen whether the response to treatment varies from group to group.

Pathogenesis

Traditionally glaucoma was a disease of the optic nerve produced by an elevated IOP either directly, by mechanical means, or indirectly, by influencing blood supply. Recognition of progressive optic nerve disease coexisting with a normal IOP required reworking of hypotheses of causation. Today there is a wide spectrum of ideas on causation. At one extreme lie the 'mechanists' who consider normal pressure glaucoma to be a disease where the IOP is 'too high for the eye'. In support of this hypothesis is the concept that the laminar architecture is less well developed, and therefore less supportive in these eyes[20]; secondly that the collagen is different and, again, less supportive[21]; thirdly, that eyes with bilateral asymmetric disease will have a higher tension in the more advanced eye.[22 23]

The other extreme is that the optic atrophy and the IOP are manifestations of the same disease, but that one does not cause the other (it should be noted that the same theory is held for high tension glaucoma too!).[24 25] Evidence in favour of this is found with the discovery that eves with normal tension glaucoma may develop raised tension with time.[26]

A middle way exists too, suggesting that all open angle glaucomas be divided into 'pressure dependent' and 'pressure independent' types, distinguishable by certain haematological and rheological features.[27] It can be difficult to identify which patient will come into which category and therefore rationalise hypotensive treatment.

The question of pathogenesis is unresolved at the present time, and with this uncertainty comes concern about what treatment, if any, to give.

Management

Treatment for high tension glaucoma is given on the not unreasonable assumption that the high tension is at least partly responsible for the visual field loss and if the tension remains high the condition will progress. Treatment for these eyes is designed to halt, or at least slow, this inevitable progression.

As noted above it can be difficult to distinguish between eyes with an anterior ischaemic optic neuropathy and normal tension glaucoma until they progress. There is no point in treating a non-progressive disease. The first requirement in management, therefore is (a) to rule out 'retrolaminar' causes for the visual loss (remembering that a skull *x* ray is inadequate for this purpose and that if doubt exists as to the nature of the visual loss the patient should undergo a computed tomographic or magnetic resonance imaging scan), and (b) to have the wherewithal to follow the patient by assessing the optic disc and IOP, but most importantly by monitoring the visual fields.

Early attempts with kinetic perimetry using the Goldmann perimeter identified progression over 2–10 years of follow up in about 40–60% of eyes.[12 28] Progression, when it occurred, often did so in a stepwise fashion (also noted in eyes with high tension glaucoma).[29] The use of computer assisted static perimetry, especially with linear regression analysis of individual retinal test locations, allowed the identification of progression more quickly and detected it more often in these eyes.[30 31] Therefore the patient should be followed with 4–6 monthly computer assisted perimetry and the visual fields analysed by linear regression analysis of each retinal point. Using this technique it is reasonable to watch the patient without treatment until evidence of progression occurs. At least 12 months of sequential perimetry may be needed for this purpose.

The treatment given to eyes with low tension glaucoma has traditionally been standard glaucoma therapy, medical laser, or surgery.[32] In a recent review of the results of such an approach Gjiessen concluded that neither medical nor laser therapy could be relied upon to produce a significant or worthwhile lowering of IOP.[19] If IOP lowering was needed for these eyes they should undergo fistulising surgery.[19 33] This will produce a significant lowering of the IOP; what remains undecided is the effect it has in preventing further visual field loss.[33]

To date there have been no published comparative trials comparing surgery versus no surgery. Anecdotal and case reports have been optimistic.[34] In a review of their long term results deJong and colleagues in Amsterdam concluded that surgery had a protective effect.[35] At Moorfields Eye Hospital an ongoing within patient prospective study has been underway for the past 5 years. In this study one eye of 14 patients with bilateral progressive low tension glaucoma was operated upon. This resulted in a significant lowering of IOP. These patients have been followed for a mean of 46 and a minimum of 29 months. To date there has been no difference in the number of significantly worsening retinal test locations, nor in the rate of loss.

Surgery may still offer some protection against further visual loss. The Amsterdam results suggest it, the Moorfields results, so far, do not. However the mean follow up of 2·5 years is a comparatively short period of time, and the IOPs achieved, in the low 'teens, may still have been too high. The Glaucoma Foundation is coordinating a multicentre study to answer these questions. Faced with a patient having progressive normal pressure glaucoma it is still justifiable to offer fistulising surgery as an option, at least to one eye. If electing to take this route, consideration should be given to the use of 5-fluorouracil, particularly if the patient is young, black, or has already received long term topical antiglaucoma treatment before.[36 37]

What options exist other than surgery? Recently attention has been drawn to the above average incidence of migraine and associated peripheral vasospasm in patients with normal tension glaucoma.[38–41] It has been suggested that these eyes may suffer from focal arteriolar constriction in the optic nerve, with subsequent optic atrophy.[42] Calcium channel blockers, by causing smooth muscle relaxation, have been put forward as a long term treatment in such cases.[43] The calcium channel blockers in current use, such as nifedipine (Adalat), can cause significant hypotension and could be contraindicated in many of our patients for this reason. 'Second generation' drugs such as lisinopril may prove easier to use. This approach, though of theoretical value, has yet to be shown to delay let alone halt progression of this disease.

R A HITCHINGS

Moorfields Eye Hospital,
City Road,
London EC1V 2PD

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British Journal of Ophthalmology 1992; **76**: 497–498

CASE REPORTS

Congenital nystagmus: rebound phenomenon following removal of contact lenses

Avinoam B Safran, Yvan Gambazzi

Abstract

Symptoms resulting from congenital nystagmus can be significantly reduced by wearing corneal contact lenses. A 90 minute therapeutic trial with contact lenses was performed on a 20-year-old affected patient and produced a beneficial effect. Upon removal of the lenses however the patient showed a transient rebound phenomenon with oscillopsia lasting about 20 minutes. This phenomenon, although it might be expected in theory, has apparently not previously been observed either because it is rare or because in most patients it is not clinically apparent. The purpose of this report is not to discourage treating patients with congenital nystagmus by means of contact lenses, but rather to draw attention to the occasional occurrence of such a rebound phenomenon and to discuss its theoretical significance.

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It is well established that alteration in visual function resulting from congenital nystagmus can be reduced by fitting corneal contact lenses.[1-4] We performed a successful therapeutic trial on a 20-year-old affected woman. When the contact lenses were removed however the patient experienced transient oscillopsia. To our knowledge such a rebound phenomenon has not previously been reported in this condition.

Case report

A 20-year-old woman was referred for evaluation of congenital nystagmus. She showed 20/20 visual acuity in both eyes, large amplitude congenital nystagmus, and no oscillopsia. The null point was in the primary position. No changes were noted in the anterior segment or the fundi. Because of a history of changes in visual discrimination in various conditions, including anxiety and poor background illumination, she was offered a therapeutic trial of contact lenses. She was fitted with therapeutic contact lenses from Essilor. The trial lasted 90 minutes and resulted in improvement of visual comfort. Two treating physicians and an accompanying person observed a reduction in nystagmus amplitude.

Immediately after removal of the contact lenses however, the patient complained of dizziness and oscillopsia. She tried to reduce these symptoms by closing her eyes. The phenomenon was intense for about 5 minutes, and some discomfort persisted for a further 15 minutes. Though evaluation of the nystagmus was limited by the patient's dizziness the amplitude of the ocular oscillation appeared to observers to be increased at the time when she complained of oscillopsia. The patient declined further attempts to fit contact lenses.

The Neuro-ophthalmology Unit, Department of Ophthalmology, Geneva University Hospital, Geneva, Switzerland
A B Safran
Y Gambazzi

Correspondence to:
Professor Avinoam B Safran,
Neuro-ophthalmology Unit,
Geneva University Hospital,
1211 Geneva 4, Switzerland.

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Discussion

In individuals with congenital nystagmus vision remains close to normal[5] unless additional abnormalities, such as albinism, are present.[6] However visual function can be disturbed when the nystagmus is increased as a result of anxiety or fixation effort[7] – for example, with poor ambient illumination or when attempting to fixate using lateral gaze because of an eccentrical null point. Rarely alteration in vision of affected patients presents as oscillopsia. This has been reported to occur in various circumstances – for example, while artificially stabilising images upon the retina.[8] Changes in nystagmus waveform might account for the occurrence of oscillopsia in these individuals.[9 10]

Numerous techniques have been suggested for improving the visual capabilities of affected patients. These include prisms,[5] surgery,[11] auditory biofeedback,[12] and corneal contact lenses.[1-4] Currently there is a strong trend towards attempting to treat such patients with contact lenses,[3 4] and these often result in dampening of the congenital nystagmus. This phenomenon might be due to sensory feedback from movement of the edge of the lens against the inside of the lids, since topical anaesthesia was shown to result in suppression of the beneficial effect of contact lenses in this condition.[3]

When first fitted with contact lenses our patient experienced relief from her visual disturbance. Upon removal of the lenses she showed a transient episode of oscillopsia. This pattern of events strongly suggests a rebound phenomenon, which is a common event occurring when a factor influencing the biological balance is suppressed, and results from the dynamic nature of the cybernetic balance process.

Rebound phenomena have been reported in normal individuals, including in ocular motor control mechanisms, as exemplified by the observation of rebound nystagmus in healthy subjects.[13] Rebound phenomena are amplified in certain conditions such as cerebellar dysfunction, as demonstrated by the Holmes rebound test,[14] or by the occurrence of marked rebound nystagmus in patients with cerebellar disorders.[15 16]

Thus, in our patient, relieving the dampening effect of contact lenses on congenital nystagmus might have induced a rebound phenomenon consisting of a transient increase in nystagmus intensity and/or a change in the foveation period. The observation of such events in our patient, but not in other treated patients, was probably due to the rarity of the phenomenon. However, the phenomenon may in fact be common, but usually occurs with minimal intensity and thus escapes notice. This appears to happen in the case of rebound nystagmus occurring in normal subjects.[13] With congenital nystagmus intra-individual variation in the intensity of rebound phenomena may be due to a number of factors, including psychological ones, especially as anxiety has been shown to influence the amplitude of congenital nystagmus.[7]

Other possible causes of the induction of oscillopsia in our patient were also considered, including those related to refractive changes or behavioural disorders. However they were not considered plausible. Indeed, changes in nystagmus did not result from an increase in fixation effort due to alteration in refractive correction because the contact lenses used in this patient were plano. The possibility that the patient's complaints were not organic in nature cannot be excluded, but there was no indication to favour such a hypothesis. If the patient was hysterical in character this would more probably have shown up while she was wearing contact lenses not after their removal.

Recording the eye movements both before and immediately after removal of contact lenses might have provided additional information. However our patient's refusal to undergo further attempts at treating the nystagmus with contact lenses precluded such an evaluation. In any event the variation which usually occurs during the recording of congenital nystagmus in relation to anxiety or fixation efforts, as well as the transient nature of the rebound, may make it difficult to record such a phenomenon.

Our report is not intended to discourage clinicians from attempting to treat patients suffering from congenital nystagmus with contact lenses since this treatment appears to be effective in many cases. Moreover, the rebound phenomenon which we describe is apparently rare and, when it occurs, is not a serious or long-lasting side-effect of the treatment. Our purpose is rather to draw attention to the possible occurrence of rebound in congenital nystagmus following removal of contact lenses, and to its theoretical significance.

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Behçet's disease: activated T lymphocytes in retinal perivasculitis

David G Charteris, Caroline Champ, A Ralph Rosenthal, Susan L Lightman

Department of Clinical Science, Institute of Ophthalmology, 17–25 Cayton Street, London EC1V 9AT
D G Charteris
S L Lightman

Leicester Royal Infirmary, Leicester LE2 7LX
C Champ
A R Rosenthal

Correspondence to:
Mr D G Charteris, Manchester Royal Eye Hospital, Oxford Road, Manchester M13 9WH.

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Abstract

A 38-year-old man who died from systemic Behçet's disease had previously suffered from severe, recurrent bilateral retinal vasculitis, and anterior uveitis for 10 years. Immunopathological examination of the eyes postmortem revealed marked hyaline thickening of the retinal and optic nerve vessels. The vessels had an intramural and perivascular infiltrate of T lymphocytes which stained positively for CD4 and IL2 receptor surface markers. Small numbers of cells in the optic nerve head, retinal vascular endothelium, and retinal pigment epithelium were HLA DR positive.

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Behçet's disease is a multisystem inflammatory disorder mediated by an occlusive vasculitis.[1] The disease is characterised by aphthous mouth ulcers, genital ulcers, and ocular inflammation. Ocular manifestations of Behçet's disease, which occur in 70–85% of cases, are a recurrent iridocyclitis, retinal vasculitis, and retinal ischaemia. These frequently lead to cystoid macular oedema and optic atrophy resulting in profound visual loss.

Alterations in complement components in aqueous humour[2] and peripheral blood[3,4] led to the theory that immune complex deposition caused the immunopathology in Behçet's disease. Studies of tissues involved in the disease process[5-8] have however shown that it is predominantly T lymphocytes which infiltrate the affected tissues. This suggests a central role for cell-mediated immunity in the disease process.

Histopathological examination of enucleated eyes from patients with Behçet's disease reveals a non-granulomatous uveitis, retinal vasculitis, and infarction.[9,10] This paper documents the findings of a pathological and immunohistochemical study on eyes obtained postmortem from a patient who died from Behçet's disease.

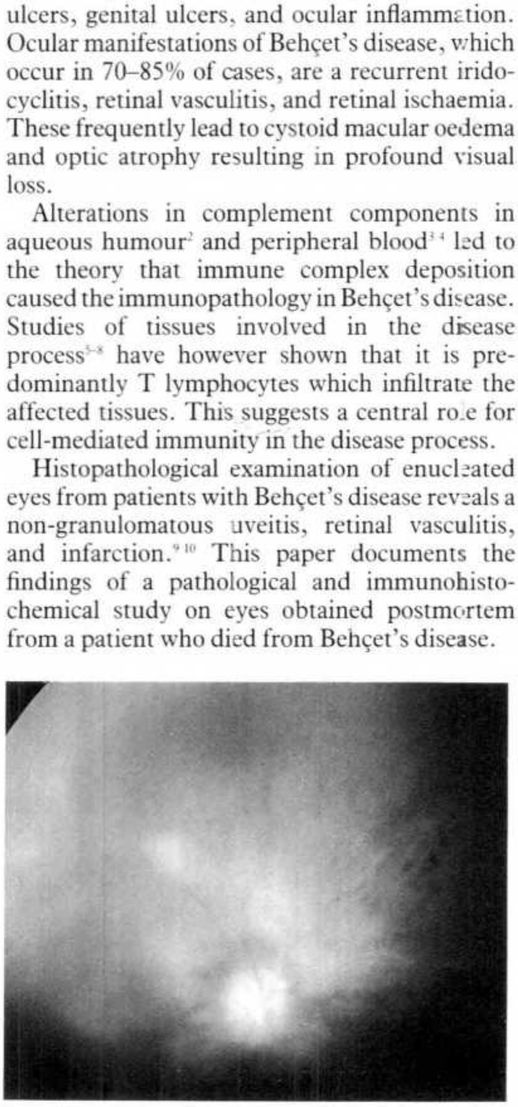

Figure 1 Right optic fundus 3 months prior to death. The optic disc is oedematous with surrounding retinal haemorrhages and patches of retinal oedema.

Case report

A 29-year-old man was diagnosed as having Behçet's disease in 1981 on the basis of a 3 year history of recurrent retinal vasculitis, anterior uveitis, and oropharyngeal aphthous ulcers. These manifestations were controlled initially by high dose oral prednisolone. Between 1985 and 1990 his systemic condition deteriorated with the development of erythema nodosum, multiple joint symptoms, and meningoencephalitis which resulted in epilepsy. Treatment with chlorambucil, cyclosporin, cyclophosphamide, and azathioprine failed to halt the progression of the disease. The patient died in October 1990 from pneumonia secondary to raised intracranial pressure due to central nervous system involvement which failed to respond to treatment.

From 1981 until his death he had recurrent episodes of anterior uveitis (with hypopyon) and retinal vasculitis in both eyes. This resulted in retinal ischaemia and visual loss despite systemic immunosuppression and orbital steroid injections. A dense cataract developed in the left eye early in the disease process, but cataract extraction was not considered justified because of the advanced retinal ischaemia. At his last ophthalmic review 3 months prior to death he had no light perception in either eye. The right optic disc was swollen and surrounded by multiple retinal haemorrhages and retinal oedema (Fig 1). The retinal vessels in the right eye were sheathed. There was no view of the left fundus due to the lens opacity. Anterior uveitis was minimal in both eyes. Intraocular pressures were normal throughout the course of the disease. Immunosuppressive treatment at the time of death was high dose systemic prednisolone, azathioprine, and cyclophosphamide.

Materials and methods

Consent was obtained for full postmortem examination including examination of the eyes. Both eyes were removed, the right globe was fixed in 4% glutaraldehyde and the left globe was frozen in OCT (Shandon, Runcorn). Horizontal sections, through the pupil and optic nerve, of both globes were cut at 6 μm thickness. Initial staining was carried out using haematoxylin and eosin. Sections of the frozen (left) eye were stained immunohistochemically using the avidin-biotin-complex (ABC) method (Vector, Peterborough). Briefly, slides were fixed in acetone, endogenous peroxidase activity quenched and blocked using normal serum. Primary monoclonal antibodies (Table 1) were applied for 30 minutes at appropriate concentration, biotinylated secondary antibody and ABC complex were subsequently applied following washing in phosphate-buffered saline.

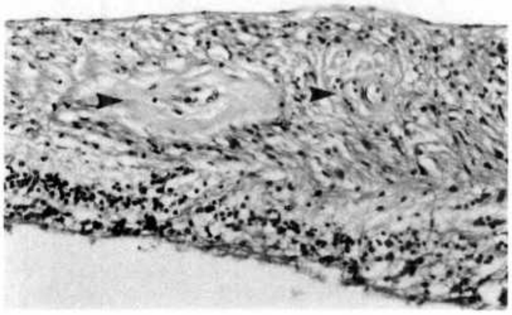

Figure 2 Disorganised, detached retina of the left eye showing marked hyaline thickening and luminal narrowing of retinal vessels (arrowheads) with intramural inflammatory infiltration. H and E ×140.

Slides were then developed in amino ethyl carbazole to give a red final reaction product and counterstained in haematoxylin.

CONTROLS

Sections of each level examined were stained immunohistochemically as above with the omission of the primary monoclonal antibody to provide negative controls. Frozen sections from a normal eye were stained with each monoclonal antibody to demonstrate the distribution of each cell type in uninflamed ocular tissue. Known positive tissues (inflammatory eyelid or orbital lesions) were stained simultaneously using each primary monoclonal antibody to verify the effectiveness of the staining procedure.

Results

Pathological examination of the brain revealed widespread cerebral oedema with gyral flattening but no evidence of coning. Histologically, there was no active cerebral vasculitis.

Table 1 Primary monoclonal antibodies

Antibody	*Source*	*Specificity*
T3	Dako*	Pan T lymphocytes
T4	Dako	CD4+ T lymphocytes
T8	Dako	CD8+ T lymphocytes
L 26	Dako	B lymphocytes
IL2R	Dako	Interleukin 2 receptor
MAC 3	Dako	Monocytes/macrophages
HLA DR	Dako	HLA DR antigen expression

*High Wycombe, UK

The pathological appearances were similar in the right and left eyes. There was a mild lymphocytic infiltrate of the iris and ciliary body; this extended into the choroid where there were scattered lymphocytes in all sections examined. The retinas showing loss of ganglion cells and photoreceptors. The retinal vessels were patent but showed marked hyaline thickening of their walls (Fig 2). There were infiltrating lymphocytes within this hyaline tissues. The retinas were focally detached with underlying patches of subretinal fluid. In the right eye the optic nerve head was oedematous and there was a lymphocytic perivasculitis of the central retinal vessels extending into the nerve. The left optic nerve head was not oedematous but had a perivasculitis. Both optic nerves had moderate loss of neural tissue.

Positive immunohistochemical staining for T lymphocytes was seen in cells in the retinal vessel walls and surrounding the optic nerve head vessels (Fig 3). These cells also stained positively with the CD4 monoclonal antibody; however the small number of cells present precluded counts of T cell subset ratios. The perivascular T cells also stained positively with the IL2 receptor monoclonal antibody. Lymphocytes in the iris, ciliary body, and choroid were also positive for the T lymphocyte monoclonal antibody although they were generally IL2 receptor negative. No CD8+ T lymphocytes or B lymphocytes were found. A few cells in the hyalinised vessel walls were positive for the macrophage primary antibody. No neutrophils were identified in the vasculitic lesions.

A small number of stromal cells of fibroblastic morphology in the optic nerve head and retinal vascular endothelial cells were HLA DR positive. There were also numerous HLA DR positive cells in the retinal pigment epithelium.

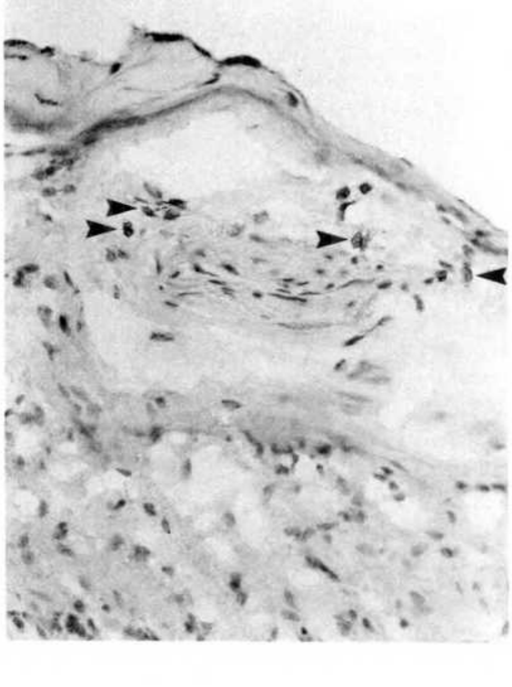

Figure 3 Optic nerve head of left eye showing CD3 positive T lymphocytes (arrowheads) around hyalinised vessel. CD3 (pan-T cell) monoclonal antibody, haematoxylin counterstain. ×260.

CONTROLS

There was minimal background staining in sections where the primary antibody was omitted. In the normal eye there were very occasional cells in the choroid which were positive for the T lymphocyte and CD4 monoclonal antibodies. There were also small numbers of cells in the choroid which were HLA DR positive. All other antibodies were negative.

Discussion

The ocular inflammation in Behçet's disease is characterised by a severe occlusive vasculitis. Most of the eyes examined pathologically from patients with Behçet's disease have been removed because of secondary complications and reflect the end stage pathology of the condition.[9,10] The eyes we have studied were obtained postmortem and were not removed because of complicating pathology. This tissue therefore, although subject to modification by immunosuppressive therapy, gives a better picture of the ongoing immunopathology of the condition. A perivascular lymphocytic infiltrate and marked thickening of the vascular walls was seen in the retinal and optic nerve vessels of both eyes and the lymphocytes in and around the vessel walls were identified as T cells. The perivascular lymphocytes in ocular sarcoidosis[11] and pars planitis[12] have also been identified as T cells.

A proportion of the T cells found in the perivascular infiltrates and within the vessel walls stained positively with CD4 and IL2 receptor monoclonal antibodies. The IL2 receptor expression of these cells is remarkable since the patient was heavily immunosuppressed at the time of death and demonstrates the difficulty in treating adequately the severe vasculitis seen in Behçet's disease.

The role of T lymphocytes in the tissue pathology of Behçet's disease has been demonstrated in immunohistopathological studies of tissues from other affected sites.[5-8] The identification of T cells as the predominant cell type in the ongoing vascular lesions in the eye supports the view that cell-mediated immune mechanisms are responsible for the tissue damage seen in this condition. The absence of B lymphocytes and neutrophils is evidence that humoral immune mechanisms do not play a major role in the ocular immunopathology.

The expression of the MHC class II antigen HLA DR by retinal pigment epithelial cells and vascular endothelial cells has been described in other forms of intraocular inflammatory disease.[11-14] Aberrant expression of MHC class II antigens has been demonstrated on endogenous tissue cells in conditions thought to be of autoimmune aetiology,[15,16] and it has been suggested that these cells may play a role in antigen presentation to infiltrating CD4+ T lymphocytes[17] such as those demonstrated in this study.

Successful immunotherapy of intraocular disease will depend on targeting specific cellular components involved in the pathogenesis of the tissue destruction. The demonstration in this study activated CD4+ T cells in the vasculitic lesions in ocular Behçet's disease suggests that future treatment directed at the down-regulation of the effects of CD4+ T cells would be beneficial in controlling the severe ocular inflammation.

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Sudden blindness in a child: presenting symptom of a sphenoid sinus mucocele

I Casteels, E De Loof, P Brock, M Jorissen, L Dralands, L Missotten, G Wilms

University Hospitals KU Leuven, B-3000 Leuven, Belgium

Department of Ophthalmology
I Casteels
L Dralands
L Missotten

Department of Pediatrics
E De Loof
P Brock

Department of Oto-rhino-laryngology
M Jorissen

Department of Radiology
G Wilms

Correspondence to:
Dr I Casteels, Department of Ophthalmology, Hospital for Sick Children, Great Ormond Street, London WC1N 3JH.

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Abstract

A 10-year-old girl with no nasal or respiratory symptoms developed a headache lasting a few hours. The next day she became totally blind in the right eye and over 5 days vision in the left eye deteriorated to bare light perception. The diagnosis of a sphenoid sinus mucocele was made radiologically and drainage via an endonasal sphenoidectomy produced 12 ml of brownish fluid. Endoscopic biopsy of the wall confirmed the diagnosis of a mucocele. Steroid treatment was given postoperatively, but vision recovered to 6/12 in the left eye only. The importance of urgent clinical diagnosis and treatment is stressed.

(*Br J Ophthalmol* 1992; **76**: 502–504)

Mucoceles of the paranasal sinuses were first described by Langenbeck in 1820 under the name hydatides but it was Rollet in 1909 who suggested the name of mucocele.[1-3] The majority originate in the frontal and anterior ethmoidal sinuses.[4]

Sphenoid sinus mucocele was first reported by Berg in 1889.[5] The presenting symptoms are variable and depend on the direction of extension into neighbouring structures. Mucocele growth is insidious but they may present acutely with neurological symptoms after expansion and bone destruction. It is a very rare condition in children and diagnostic clues are based on the history, signs of orbital and optic nerve disease and are confirmed by radiological examination.

Case report

A 10-year-old girl was referred by her ophthalmologist because of bilateral blindness which developed over the previous 5 days. One week earlier she had complained of severe frontal headache for several hours. The next day she became aware of complete blindness in the right eye, followed by loss of vision in the left eye over the next 5 days. On admission there was no light perception by the right eye and minimal light perception by the left eye, with complete loss of colour perception. Pupillary light reactions were absent in both eyes and the pupils were dilated. Visual field testing was not possible. On funduscopy bilateral symmetrical pallor of the temporal parts of the optic disc without oedema could be seen. The child was in good general health. She did not suffer from any nasal or respiratory problems and general clinical and neurological examination were normal. Routine laboratory testing did not reveal any abnormalities. Analysis of the pituitary gland hormones proved later to be normal.

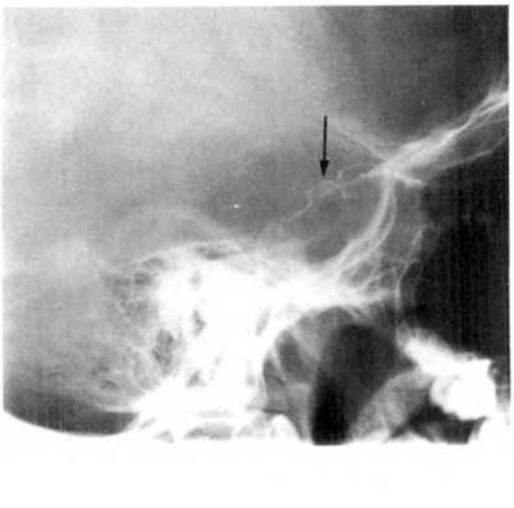

Figure 1 Lateral plain skull radiograph. Homogeneous opacification of the sphenoid sinus and abnormal configuration of the sella turcica.

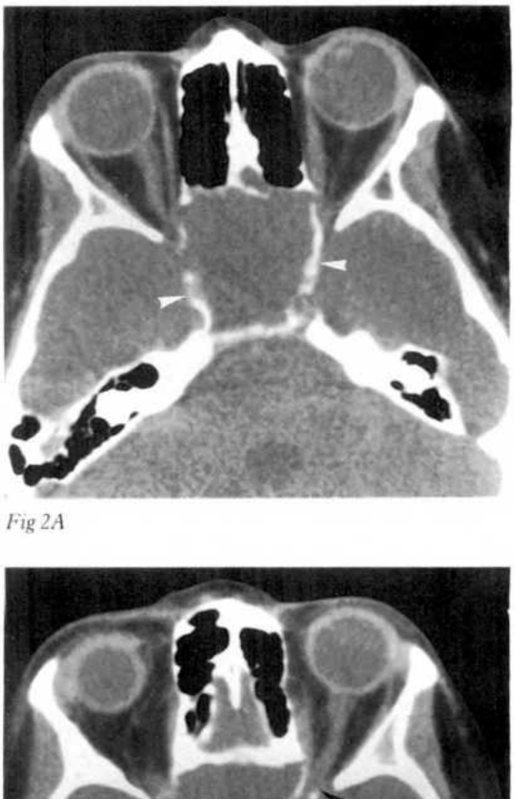

Fig 2A

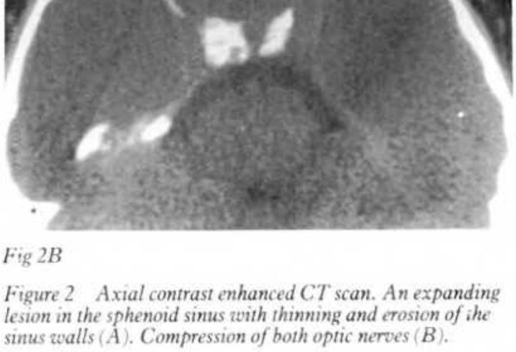

Fig 2B

Figure 2 Axial contrast enhanced CT scan. An expanding lesion in the sphenoid sinus with thinning and erosion of the sinus walls (A). Compression of both optic nerves (B).

Figure 3 Coronal contrast enhanced CT scan. Compression of the optic nerves (A), ballooning of the sellar floor (B), and normal optic chiasm and pituitary gland (C).

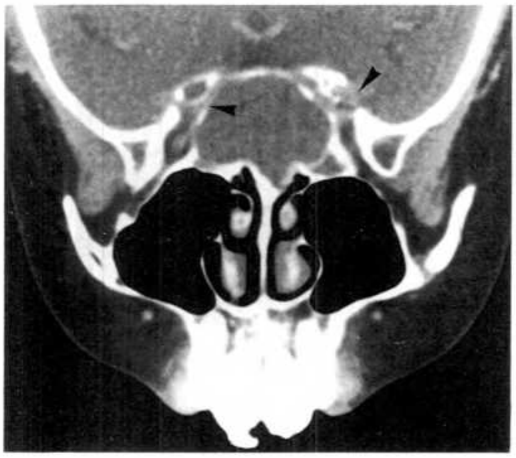
Fig 3A

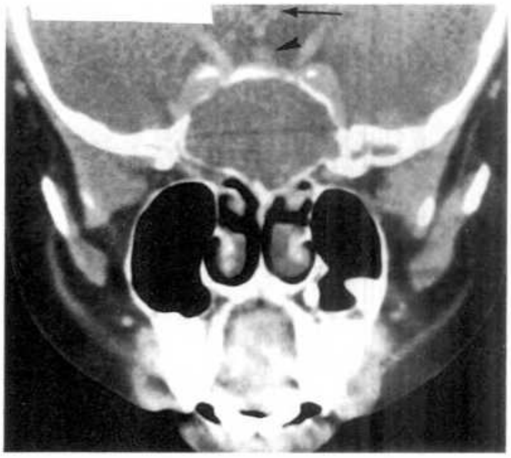
Fig 3C

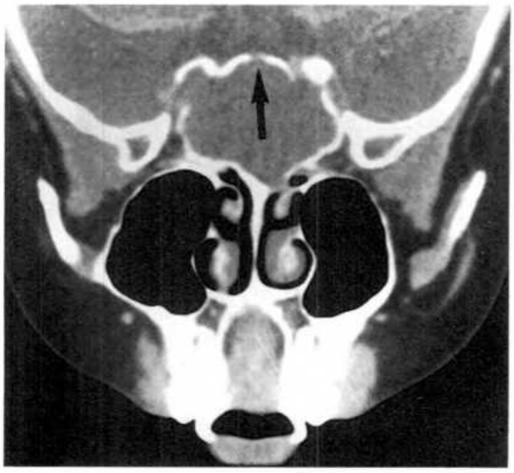
Fig 3B

Plain skull radiographs demonstrated a homogeneous opacification of the sphenoid sinus (Fig 1), the sella turcica could not adequately be identified, and ballooning of the sellar floor was suspected. Axial computed tomography (CT) demonstrated a mass lesion in the sphenoid which was approximately isodense with the grey matter of the brain, with thinning and erosion of the sinus walls (Fig 2A, 2B). There was upward erosion with ballooning of the sella and bilateral compression of the optic nerves in the optic canals. The pituitary gland and chiasm appeared normal (Fig 3A, 3B, 3C). A bone marrow smear was normal. As the diagnosis of a sphenoid sinus mucocele was suspected endonasal sphenoidectomy was performed with aspiration of about 12 ml of yellow brown fluid. On endoscopic examination the cavity was lined by a thin mucosal layer without irregularities. Drainage of the sinus was continued for 3 days. Biopsy of the cavity wall confirmed the diagnosis of a mucocele.

Treatment with antibiotics and corticosteroids was initiated. Dexamethasone 2·5 mg/kg, flucloxacillin 70 mg/kg, and amoxycillin 50 mg/kg were given intravenously at day 2 and day 3. For the next 2 weeks she was treated with dexamethasone 35 mg/day, flucloxacillin 500 mg/day, and amoxycillin 375 mg/day. The corticotherapy was tapered to zero gradually over the next 2 months. A CT scan after 1 month showed an empty sphenoid sinus. Vision in the left eye recovered to 6/24 after 2 months and 6/12 after 3 months. On visual field testing there was a residual relative paracentral scotoma at the left eye. The right eye did not recover; there is no light perception. Complete atrophy of the right optic disc and temporal pallor of the left optic disc were evident.

Discussion

In young children, slowly progressing visual loss due to chiasmal or optic nerve disease usually presents late because a child compensates quite well a partial or unilateral visual loss. Only when a substantial and bilateral defect has developed do visual problems become obvious.[6]

In our patient the bilateral temporal optic atrophy on admission suggested a longstanding lesion. Blindness was presumably precipitated by a critical increase in the size of the lesion with sudden deterioration of optic nerve function. Lundgren[4] described 60 adults with sphenoid mucocele with unilateral and bilateral blindness. A recent review of the literature describes the presenting signs and complications of a sphenoidal sinus mucocele in 130 cases.[7] The youngest patient was 8 years old and presented with exophthalmos.[8] As these lesions grow very slowly sphenoid sinus mucoceles are rare in children,[9] and permanent visual loss caused by one is probably exceedingly rare.

A mucocele is a collection of mucus enclosed in a sinus epithelium within an air sinus, resulting from the obstruction of the ostium by inflamed mucosa. Secondary causes of obstruction are a cystic degeneration of an epithelial mucus gland or a cystic degeneration of an inflammatory polyp. However there is a large group in which no cause has been found.[10]

The presenting signs and symptoms of a mucocele originating from the sphenoid are variable and depend on the direction of extension towards neighbouring structures.[11-12] Headache, facial pain, anosmia, ocular palsy, and visual failure in adults are common, the most consistent being headache, which is usually frontal or retro-orbital. Pupil sparing third nerve palsy is described[13] but paresis of the fourth and sixth cranial nerve is less common; it is caused by the expansion of the mucocele into the cavernous sinus or the superior orbital fissure. Exophthalmos is caused by an invasion in the ethmoidal sinus and lateral displacement of the medial orbital wall. Elevation of the chiasmal sulcus does not produce optic chiasmal field defects because of the lack of compression on the chiasm. Hypopituitarism has been described.[14] Other symptoms are rhinorrhoea associated with nasal polyposis. Our patient complained of a short period of frontal headache 1 day before the visual loss at the right eye, but the absence of nasal or respiratory problems is noteworthy.

Mucoceles of the sphenoid sinus must be differentiated from the following lesions: hypophyseal tumours, craniopharyngioma, meningioma and glioma in the neighbourhood of the sella turcica, intracranial chordoma, dysgerminoma and cholesteatoma, and finally tumours arising from nasopharynx, sinus, and base of the skull. Erosion of the sellar floor with visual field defects and endocrine disturbances can simulate a pituitary tumour. However an important feature in the differential diagnosis is the absence of a bitemporal field defect in a mucocele because there is no direct compression on the chiasm.[4] Visual field testing during recovery in our patient showed a paracentral scotoma in the left eye. Differentiation between sphenoid mucoceles and pyoceles or cysts is difficult but the surgical indications and approach are the same.

The sphenoid sinus is difficult to examine clinically and reliance on plain skull radiographs, brain CT, or nuclear magnetic resonance imaging is necessary.[15] Mucoceles are associated with bone destruction. Bony abnormalities are demonstrable on plain radigraphs using the submentovertical and lateral views. They can be confirmed with a CT scan.[10] Additional anatomopathological examination of the content confirms the presumed diagnosis of a mucocele.

Sphenoid surgery was first introduced by Schloffer in 1885.[16] Surgical treatment consists usually of transnasal sphenoidectomy and sinotomy. Other possibilities are transethmoidal, transantral, transpalatal, or trans-septal. Early surgical intervention is essential: if surgery is delayed for more than 6 to 10 days after the start of visual loss and if there is optic atrophy the visual prognosis seems to be poor. Our patient was operated 6 days after she became aware of profound visual loss in the right eye and 1 day after loss of vision in the left eye. After adequate surgical intervention and treatment with corticosteroids the vision recovered in the left eye to 6/12.

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Actinomycosis of the orbit

Timothy J Sullivan, G W Aylward, J E Wright

Abstract

Actinomycosis is a very rare cause of orbital abscess usually attributable to direct spread from adjacent structures. A case of actinomycosis of the orbit is presented, which was treated as orbital pseudotumour for 3 months before progression of signs and symptoms, despite high dose steroids, led to the diagnosis being reconsidered.

(*Br J Ophthalmol* 1992; 76: 505–506)

Case report

A 42-year-old Caucasoid woman was referred to a neurologist with a 3 week history of a right temporal headache, radiating around her right eye. For the past 5 years the vision in the right eye had been counting fingers, because of a macular scar of unknown aetiology.

Computed tomography (CT) of the head failed to show any abnormality. She was placed on oral dexamethasone to relieve the pain and made a dramatic symptomatic response. As the steroid dosage was reduced she developed right periocular tenderness, proptosis, and eyelid swelling.

She was admitted for further investigation. The erythrocyte sedimentation rate (ESR) was 70 mm/h, there was a mild leucocytosis, and lumbar puncture was normal. While she was an inpatient her right second upper molar tooth fractured spontaneously; infection of the affected tooth was not evident on dental examination or *x* ray. Orbital CT showed a small ill-defined mass, isodense with brain, in the posterolateral part of the right orbit (Fig 1). She was seen by an ophthalmologist, who commenced antibiotics in view of the elevated ESR and leucocytosis. Another ophthalmologist believed she had orbital pseudotumour and recommenced her on high dose steroids, with another dramatic improvement. The proptosis progressed and the headache and pain returned, however, when the steroid dosage was reduced.

At the time of referral to the orbital clinic (Fig 2) the right visual acuity was reduced to counting fingers and the left eye was normal, with a visual acuity of Snellen 6/9. There was a right afferent pupillary defect and a right central scotoma.

There were 5 mm of right relative proptosis, decreased right corneal sensation, and complete loss of right ocular ductions. A small scar was present at the right macular but the optic disc and retinal vessels were normal.

At the time of orbital exploration, through a lateral canthotomy approach, an abscess (containing green pus) was encountered within the inner surgical space. The abscess was drained and the patient was given metronidazole and high dose intramuscular penicillin V with probenecid. Gram stain revealed actinomycosis and culture grew a pure heavy growth of *Actinomyces israelii*.

Three months postoperatively the patient was well with no proptosis, a moderate right ptosis, and a mild right abduction weakness.

Discussion

This rare case of orbital actinomycosis was presumed to result from direct extension of an endogenous infection of the oral cavity, probably related to the carious tooth that later fractured. Several reports of orbital actinomycosis in the older literature exist[1-6]; however we were unable to trace any recent reports of orbital actinomycosis, although one case of Tolosa-Hunt syndrome reported in 1979 was attributed to

Orbital Clinic, Moorfields Eye Hospital, London
T J Sullivan
G W Aylward
J E Wright

Correspondence to:
Mr J E Wright, Moorfields Eye Hospital, City Road, London EC1V 2PD.

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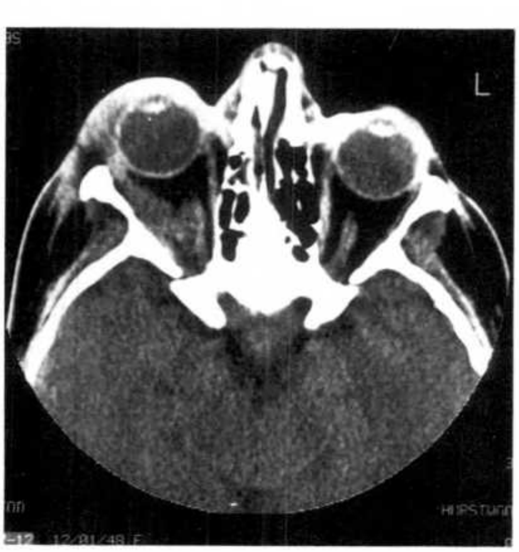

Figure 1 Orbital CT showed a small ill-defined mass, isodense with brain, in the posterolateral part of the right orbit.

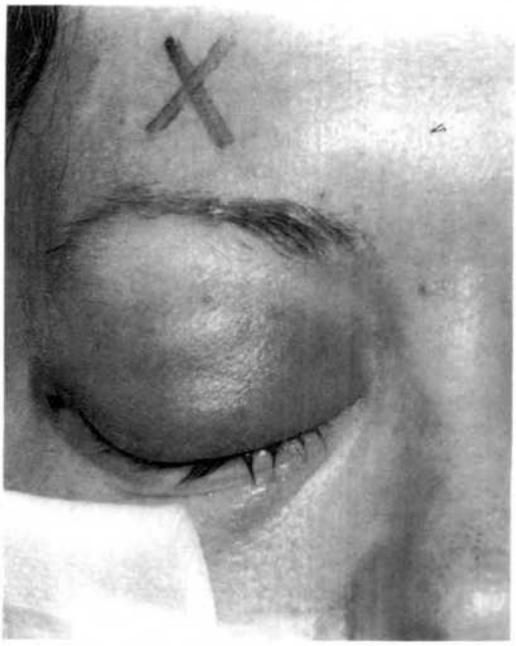

Figure 2 Prior to orbital exploration there was right proptosis and soft tissue swelling closing the right eye.

Actinomyces israelii.[7] This decrease in incidence has been attributed to better oral hygiene and the widespread use of broad-spectrum antibiotics. *Actinomyces israelii*, a higher bacteria with some fungal characteristics, forms part of the normal flora of the oral cavity.[8] Rarely the orbit may become involved secondarily from infections of the infratemporal fossa, the paranasal sinuses, or ocular adnexal structures.[9] In contrast to this case, orbital actinomycosis usually presents with painless proptosis and restricted extraocular movements. Fistulas and sinus formation may follow.[9] Treatment of actinomycosis usually consists of high dose penicillin, or clindamycin or chloramphenicol in patients with an allergy to penicillin.

This case illustrates the danger of treating patients with a presumed diagnosis of pseudotumour with a trial of steroids without prior surgical exploration and biopsy.[10-12] Such an approach often masks the true nature of the orbital lesion and delays correct therapy.[13 14]

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Phakomatous choristoma of the orbit: a case report

Brian Leatherbarrow, Jeffrey A Nerad, Keith D Carter, Jacob Pe'er, Jean Spencer

Abstract

We report the case of a 3-month-old infant with a rare phakomatous choristoma of the orbit. This lesion is believed to be a congenital neoplasm of lenticular anlage. The clinical, radiological, and histopathological findings are presented.

(*Br J Ophthalmol* 1992; **76**: 507–508)

Zimmerman first described the clinical and histopathological features of phakomatous choristoma in three infants.[1] Since then there have been six further case reports in this rare and unusual tumour.[2-7] In all these cases the lesion was described as a lower eyelid mass with occasional extension posteriorly into the orbit. In our case the lesion was located entirely posterior to the orbit septum and was confined to the anterior orbit. We present the characteristic clinical, radiological, and histopathological findings, and suggest that this lesion be added to the differential diagnosis of an anterior orbital mass in an infant.

Case report

A 9-week-old female infant was seen by an ophthalmologist with a history of a lump visible in the inferomedial aspect of the right lower eyelid (Fig 1). The lesion had been noted by the mother shortly after birth. This was associated with some swelling of the lower eyelid and epiphora. The lesion was initially thought to be a mucocele of the lacrimal sac and was treated conservatively. The lesion remained unchanged and the patient was referred to the oculoplastic clinic having undergone a computed tomography (CT) scan of the orbits.

The lesion had remained unchanged in size since it was first noted. On examination a 1 cm× 1 cm, discrete, firm, mass was palpable through the skin of the lower eyelid medially. It was situated 0·5 mm lateral to the punctum. The lesion was non-mobile, non-fluctuant, not attached to overlying skin, and there was no overlying skin discoloration. There were no abnormal pulsations and no increase in size of the lesion with a positive Valsalva manoeuvre. Pressure over the lesion was not associated with reflux of mucus or pus from the puncta. There was a mild degree of epiphora on the right side and a fluorescein dye disappearance test was prolonged. A cycloplegic refraction was per-

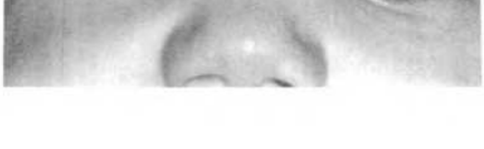

Figure 1 Photograph of the infant demonstrating a visible mass lesion in the inferomedial aspect of the right lower eyelid.

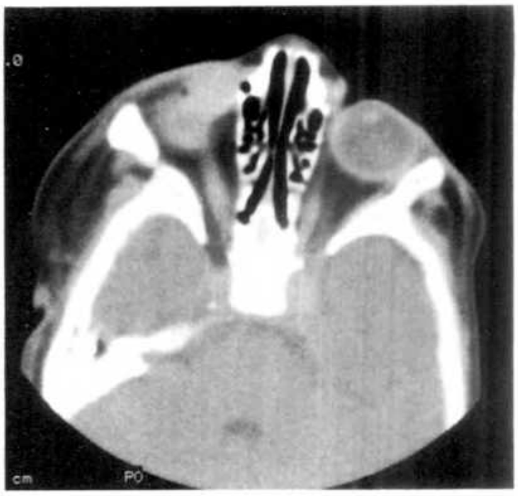

Figure 2 Axial CT scan demonstrating a discrete well-circumscribed homogeneous anteromedial lesion in the right orbit inferiorly.

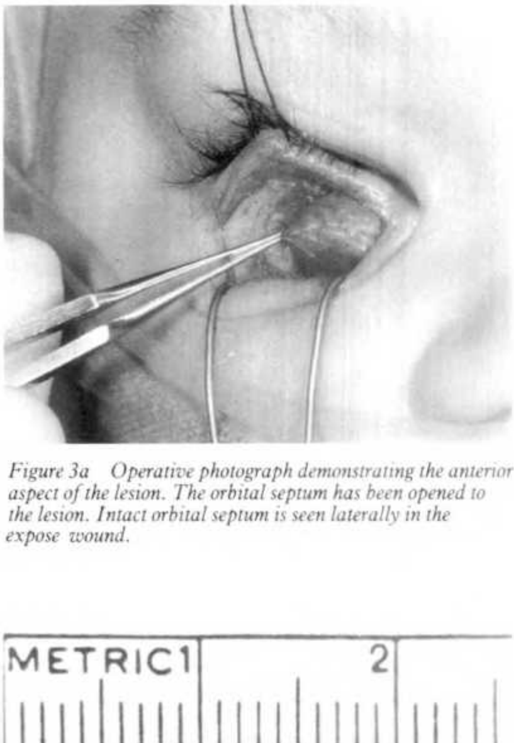

Figure 3a Operative photograph demonstrating the anterior aspect of the lesion. The orbital septum has been opened to the lesion. Intact orbital septum is seen laterally in the expose wound.

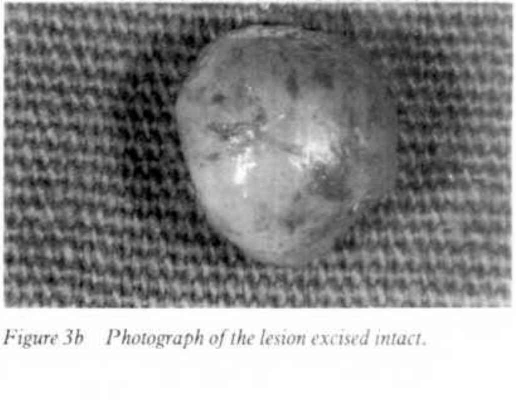

Figure 3b Photograph of the lesion excised intact.

Department of Ophthalmology, University of Iowa Hospitals and Clinics, Iowa City
B Leatherbarrow
J A Nerad
K D Carter

Department of Ophthalmology, Hadassah University Hospital, Jerusalem, Israel
J Pe'er

Private practice, Des Moines, Iowa
J Spencer

Correspondence to:
B Leatherbarrow, FRCS.

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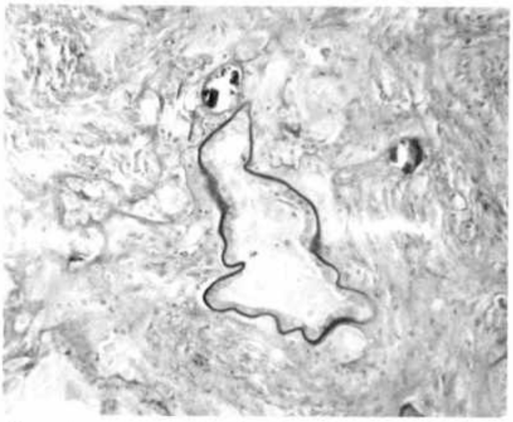

Figure 4a Low magnification photomicrograph of the lesion demonstrating islands of cuboidal epithelium embedded in dense collagenous stroma.

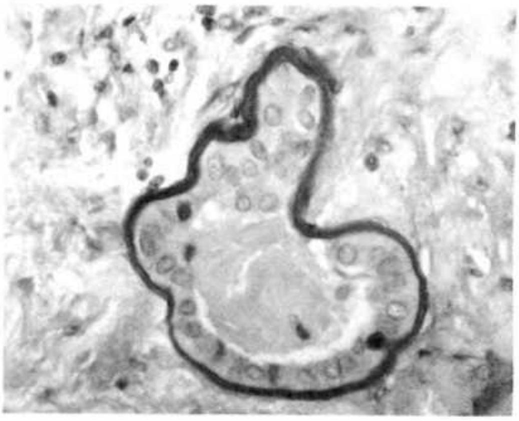

Figure 4b High magnification photomicrograph of the lesion demonstrating an epithelial island surrounding a central eosinophilic globule. The epithelial island is surrounded by thickened basement membrane material.

formed the result of which was as follows: OD+ 0·50/+2·00×120, OS+0·50.

The infant was the product of a full-term pregnancy. Delivery was normal and the baby was in good health. The remainder of the ocular examination, as well as the general physical examination, was normal.

The CT scan showed a discrete, well circumscribed, homogeneous, non-cystic anterior orbital lesion not involving the lacrimal drainage apparatus (Fig 2). There were no other orbital anomalies. Standardised echography showed the lesion to be well circumscribed and highly reflective.

A preoperative clinical diagnosis of a neoplastic lesion was made. The patient underwent an excision biopsy of the lesion via a subciliary skin incision. The lesion was found to be posterior to the orbital septum with which it was partially fused. The lesion was solid, white, and nodular with no discrete capsule (Fig 3). It was dissected from the surrounding tissues. It was adjacent to the inferior oblique muscle and did not directly involve the lacrimal drainage apparatus.

The lesion measured 10×8×4 mm. Histological examination showed the tumour to consist of dense collagenous stroma in which islands of benign cuboidal epithelium were embedded (Fig 4). These epithelial islands surrounded central eosinophilic globules, consistent with lens cortex. Some of these globules were swollen and contained benign nuclei resembling 'bladder cells' of human cataractous lenses. The epithelial islands were surrounded by thickened basement membrane material. Small foci of dystrophic calcification were also identified.

Discussion

The clinical findings of this case are similar to those of previously reported cases except that the lesion in this case was confined to the orbit.[1-7] The phakomatous choristoma always appears to be confined to the antero-inferior aspect of the eyelid/orbit medially. This location is the most consistent feature of the lesion. The close proximity of the lesion to the junction of the lacrimal sac and nasolacrimal duct was probably responsible for the infant's epiphora. This resolved spontaneously postoperatively.

It has been postulated that this lesion is due to some of the surface ectodermal cells, which are induced to form the lens plate and lens vesicle in the embryo, remaining external to the optic vesicle as the embryonic fissure closes.[7] These cells would then multiply and form the phakomatous choristoma after undergoing rudimentary differentiation. Its constant inferonasal position might also be explained by the ventronasal location of the embryonic choroidal fissure of the optic vesicle.

This is a rare and interesting tumour which has never been correctly diagnosed preoperatively in any of the previously reported cases. This case is no exception in this regard. The clinical, radiological, and echographic characteristics of this tumour together with an awareness of its existence should permit its ready differentiation from most other orbital lesions occurring in childhood in this anatomical location.

This research has been supported by an unrestricted grant from Research to Prevent Blindness, Inc, New York.

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Broken intraocular lens during cataract surgery

J N P Kirkpatrick, S D Cook

Bristol Eye Hospital, Lower Maudlin Street, Bristol BS1 2LX
J N P Kirkpatrick
S D Cook

Correspondence to:
J N P Kirkpatrick, Bristol Eye Hospital, Lower Maudlin Street, Bristol BS1 2LX.

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Abstract

A case of planned routine extracapsular cataract extraction is described where surgery was complicated peroperatively by fracture of the posterior chamber lens implant. The technique of lens implantation is discussed.
(*Br J Ophthalmol* 1992; **76:** 509)

Posterior chamber lenses are used preferentially in routine extracapsular cataract surgery in this department. Many complications may occur during or after implantation and have been reviewed in detail elsewhere.[1,2] We describe a case where breakage of the intraocular lens occurred during surgery.

Case report

A 64-year-old man with age-related cataract was admitted for routine cataract surgery to the right eye. Visual acuity was 6/36 (right eye) and 6/9 (left eye).

Under general anaesthesia a corneal section, can opener capsulotomy, nucleus expression, and cortical soft lens matter aspiration was performed. Under air a one-piece PMMA 7 mm optic posterior chamber implant (Iolab) was inserted. Despite good mydriasis and the use of a viscoelastic agent there was some difficulty in placing the superior haptic and so it was elected to dial the lens into position using a Sinsky dialling hook. After approximately 7 clock hours of dialling the haptic was still in the anterior chamber and the dialling hook had become stuck in the dialling hole at the base of the haptic. A gentle rolling action of the dialling hook resulted in fracture of the lens across the dialling hole so that the haptic became detached. The two broken pieces were removed (Fig 1) and a second intraocular lens (three-piece PMMA, 6 mm optic) was inserted with ease.

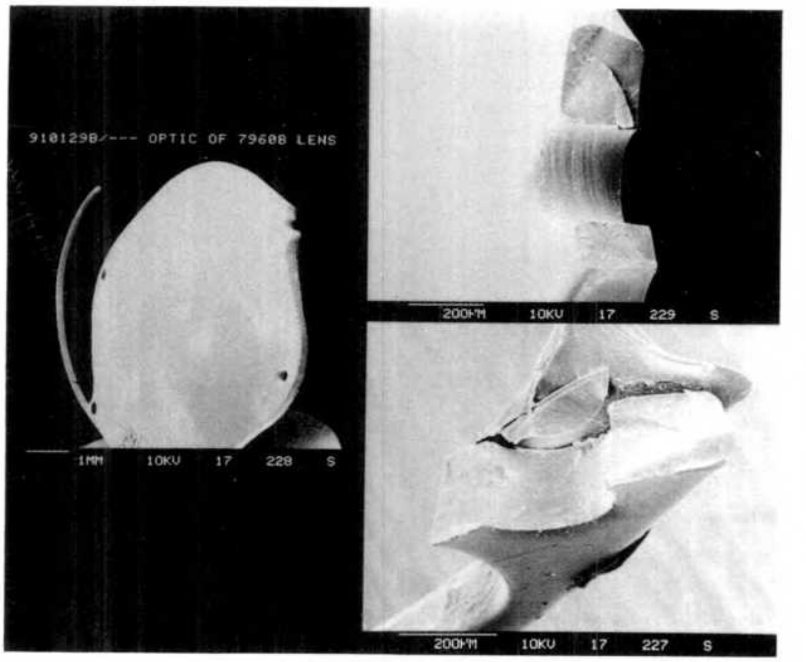

Figure 1 (left) Scanning electron micrograph of intraocular lens showing fracture across haptic base; (top right) magnified view of optic showing smooth plane of fracture: (bottom right) broken haptic with adherent debris.

The postoperative phase was uneventful and the patient achieved 6/6 vision with spectacle correction.

Discussion

No previous cases of fracture of the intraocular lens during surgery have been reported. Fractures of anterior chamber implants of one-piece and three-piece design have been reported postoperatively in cases which do not necessarily have a history of trauma.[3,4]

The cause of the fracture in this case is most likely to be due the application of a shearing force across the dialling hole by the dialling hook. The rolling action of the hook resulted in compression of the PMMA material alongside the dialling hole which was sufficient to cause fracture. The dimension of the dialling hole in this lens is 0·3 mm diameter which is somewhat smaller than that found in the three-piece lens used more frequently in this department (0·4 mm diameter). A smaller dialling hole is more likely to lead to the impaction of the dialling hook which was encountered here. There was no indication at the time of surgery that the dialling hook had any imperfection which might encourage difficult release from the dialling hole. Similarly there was no sign of pre-existing lens imperfection macroscopically.

PMMA is a relatively brittle material. The results of scanning electron microscopy on the broken lens show a sharp cleavage plane which is characteristic of the material. It has been suggested that this property may increase after the passage of time or manipulation.[1]

The use of forceps implantation rather than dialling would avoid the complication described above for lens implantation. A Rycroft cannula or a silicone rubber tipped instrument may permit lens positioning without use of the dialling hole. Finally it is suggested that the positioning of dialling holes as an integral part of the haptic base may result in shearing forces which were not taken into account at the time of lens manufacture. Repositioning of the dialling holes in intraocular lenses may avoid the complications described above.

We thank Iolab UK for their technical assistance and their agreement to this report on the case.

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LETTERS TO THE EDITOR

Cowpox virus

SIR,—In 1889 Parinaud[1] described a uniocular conjunctivitis acquired by close contact with infected animals. It was a granular conjunctivitis accompanied by swollen eyelids and a mucopurulent secretion. The parotid region was swollen and inflamed. The granulation tissue persisted for months and histologically there were epithelioid and mast cells present.

A variety of agents have been implicated – for example, cat-scratch disease, tularaemia, tuberculosis, blastomycosis, coccidioidomycosis, syphilis, and actinomycosis, etc.[2]

We report a necrotic granulomatous conjunctivitis caused by the cowpox virus, a virus closely related but not identical to vaccinia.[3] There is no recorded case of cowpox conjunctivitis occurring in the United Kingdom.

A 15-year-old boy who lived on a farm was referred with 1 week's history of swollen sore left upper and lower eyelids with inflamed conjunctiva. Initially he noticed slight erythema on the left lower lid. One day later the conjunctiva had become inflamed and both lids swollen and the GP noticed a small red spot on the lower lid and tiny blisters on the conjunctiva. The lid swelling had increased until after 7 days he could not open the eye and the left side of his face became swollen (Fig 1). There was no history of trauma to the eye and the condition had not responded to systemic antibiotics.

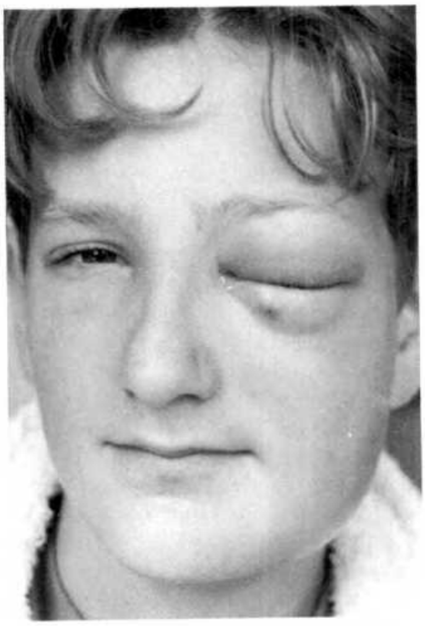

Figure 1 Appearance of patient 7 days after onset of conjunctivitis.

Using Desmarre's retractors a very chemosed conjunctiva with mucopurulent discharge on the surface was exposed. The cornea was covered by the swollen conjunctiva. A provisional diagnosis of purulent conjunctivitis with preseptal cellulitis was considered. The following day the patient was examined under general anaesthesia. Despite appearances both upper and lower lids were of normal thickness and the fornices were totally free of adhesions. The bulbar conjunctiva was approximately 7 mm thick and there was a 5 mm cuff of perilimbal necrotic conjunctiva. This was excised and an area of thickened conjunctiva was sent for histology. The cornea, the fundi, and the media were normal. Swabs were taken for viral tissue culture and smear for inclusion bodies.

A diagnosis was made of acute fulminating necrotic conjunctivitis due to herpes simplex. Two days later he developed indurated areas of bulbar conjunctiva palpated through the upper and lower lids.

The patient left hospital before further histological examination. The histological report confirmed a severe conjunctival infection with areas of necrosis and epithelioid and round cell infiltration. The indurated areas were considered to be granulation tissue with a marked fibrotic response. The tissue culture grew a cowpox virus. The carrier of cowpox virus is thought to be a domestic cat.[4]

Three months later the visual acuity was 6/6 in each eye and the bulbar conjunctiva under the superior and inferior eyelids remained swollen, red and indurated, but not tender. There was a 7 mm area of symblepharon affecting the lower lid and a 5 mm polypoid excrescence of bulbar conjunctiva in the superior temporal quadrant.

W N DUGMORE
Z M DABIR
Department of Ophthalmology,
District General Hospital,
Burnley

1 Parinaud H. Infective conjunctivitis of animal origin. *An d'Oc Sci* 1889; 252.
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Segmentation of fluorescence in the retinal microcirculation – is it a valid indicator of blood cell flow?

SIR,—We read with interest the article of Arend *et al* on the use of scanning laser ophthalmoscopy for retinal capillary blood flow studies.[1] Perifoveal capillary blood cell velocities were found to be reduced in diabetic patients compared with normal subjects. The basic assumption for the blood flow measurements was that the segmentation in the fluorescence intensity corresponded to segments of erythrocytes in the form of rouleaux formation (low fluorescence) and cell-free plasma (high fluorescence).

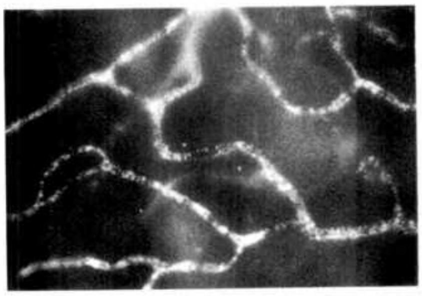

Figure 1 Retinal vascular net of a rat. Stable fluorescence of the capillary lumen by circulating microspheres of 0·022 μm in diameter. Intensity of fluorescence is not homogeneous along the vessels.

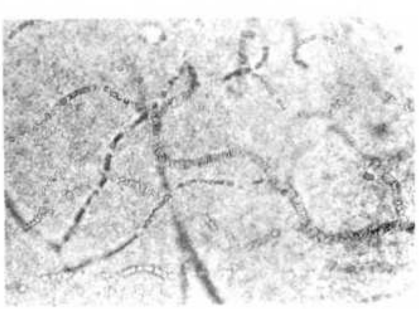

Figure 2 The same retinal location as in Figure 1 illuminated with white light to show the red blood cells. The density of the blood cells in the capillaries does not necessarily correlate with the alterations in the fluorescence intensity.

Using our vascular trichrome method[2] we noticed that segmentation in fluorescence intensity does not necessarily correspond to the erythrocytes versus plasma assumption. Figure 1 shows a retinal capillary (rat) with alterations of the fluorescence intensity along the vessel. In Figure 2 the same vessel is illuminated with white light, demonstrating that erythrocytes are seen throughout both high and low fluorescence areas. We might have regarded these findings as post mortem artefacts were they not supported by other experimental data. We recently developed a new method, named fluorescent blood cell angiography, for in vivo dynamic observation of fluorescent labelled erythrocytes in the retinal capillary net.[3] By changing the filter setting of the imaging system a conventional fluorescein angiography of the same capillary net can also be performed.

These observations were recorded on a video tape for later analysis. Using this new method we found that the fluorescein segmentation velocity in the capillary net does not necessarily correspond to the blood cell velocity. While in some capillary paths the labelled blood cell velocity did correspond to the segmentation velocity, in other capillary paths in the same retina these velocities did not correspond. Moreover, factors such as systemic blood pressure, hyperglycaemia, intraocular pressure, and capillary architecture seem to have an unpredictable effect on the ratio between blood cell flow and the phenomenon of fluorescent segmentation. In summary, we think that the scanning laser ophthalmoscope is a promising tool in future analysis of capillary blood flow. Nonetheless, capillary fluorescence segmentation has to be better understood if this phenomenon is to be used for quantitative retinal capillary blood cell flow measurements.

JOSHUA BEN-NUN
IAN J CONSTABLE
The Lions Eye Institute and Department of Surgery,
University of Western Australia,
Nedlanas, Australia

1 Arend O, Wolf S, Fung F, Bertram B, Postgens H, Toonen H, Reim M. Retinal microcirculation in patients with diabetes mellitus: dynamic and morphological analysis of perifoveal capillary network. *Br J Ophthalmol* 1991; **75**: 514–8.
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Reply

SIR,—Biomicroscopic recordings of conjunctival (Fig 1) and periungual capillaries[1,2] clearly show segmentation corresponding to erythrocytes versus plasma. From these findings and our experience in conjunctival video

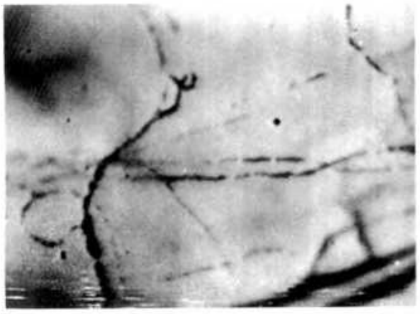

Figure 1 Conjunctival capillary vessels (magnification ×100) showing the segmentation plasma versus corpuscular formation.

angiographic observations we conclude segmentation in the fluorescence intensity corresponds to segments of erythrocytes and cell-free plasma. The figures of Ben-nun and Constable do not necessarily contradict our assumption. The segmentation of fluorescence intensity seems to correspond to packed cells. The interpretation of the postmortem findings could be clarified if the illumination was changed from white to green light. With green light illumination the contrast between red blood cells and plasma is best, owing to the maximum of absorption of haemoglobin.

The fluorescent blood cell angiography mentioned is very interesting. Those findings may clarify the interpretation of our report. Recently Tanaka *et al*[3] observed fluorescent dots in perifoveal capillaries. They proposed that these dots correspond to leucocytes and platelets in the circulating blood. We do not agree with their conclusion. They are using the automatic gain control in the set-up of the scanning laser ophthalmoscope which leads to decreased signal/noise ratio.

In conclusion, we think that our interpretation of the observed phenomenon (Fig 2) seems to be acceptable. In addition until now our method is the only one that measures flow velocities and morphological parameters in the perifoveal capillaries objectively.

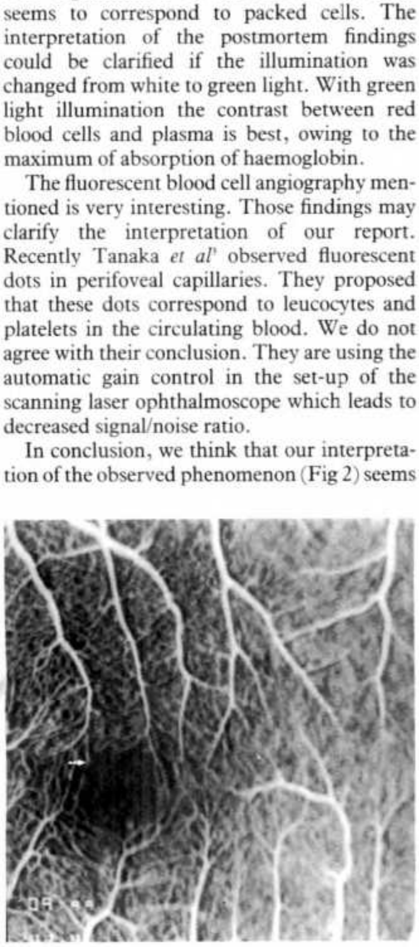

Figure 2 Perifoveal capillary network with hyperfluorescent gap (arrow) in macular capillary (modified from Wolf et al*).*

O AREND
S WOLF
*Augenklinik der Medizinischen,
Fakultät RWTH Aachen,
Pauwelsstrasse 30, D-5100
Aachen, Germany*

1 Jung F, Körber N, Kiesewetter H, Prünte C, Wolf S, Reim M. Measuring the microcirculation in the human conjunctiva bulbi under normal and hyperperfusion conditions. *Graefes Arch Clin Exp Ophthalmol* 1983; **220**: 294–7.

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Periorbital necrobiosis lipoidica

SIR,—I read with interest the case reported by Mr Lavy and colleagues.[1] An important differential and possible alternative diagnosis to that suggested which does not appear to have been considered is that of necrobiotic xanthogranuloma (NXG). This now well described condition is a non-X histiocytic disease characterised by a conspicuous dermatosis with a particular predilection for the periorbital tissues. Prior to its description in 1980 by Kossard and Winkelmann,[2] it had previously been described in a variety of ways including atypical necrobiosis lipoidica.

As in the case discussed NXG presents with painless non-pruritic papules that progress to nodules and plaques which may vary in appearance but usually have a xanthomatous element. These lesions may remain subclinical for extended periods but can pursue an aggressive course with recurrent severe ulceration of the skin lesions. These usually have pronounced telangiectasis in the ulcerative phase.

The importance of this alternative diagnosis is that NXG is invariably associated with a dysproteinaemia, usually a monoclonal paraproteinaemia of the IgG class. This may follow a benign course but malignancies, typically multiple myeloma and chronic lymphatic leukaemia, may develop. The lesions may also involve the orbit posing a potential threat to vision.[3,4]

The histopathological findings in the case described could be consistent with a diagnosis of NXG. The features found in NXG of a nonspecific lymphocytic and plasma cellular infiltrate with palisading granuloma formation, together with areas of collagen necrobiosis and giant cell formation are similar to the biopsy illustrated. More specific features of NXG would be xanthogranulomatous panniculitis, and distinct palisading cholesterol cleft formation.

In view of this, further investigation of this patient that may be warranted would include serum protein and lipoprotein electrophoresis, urinalysis for Bence-Jones protein, and a computed tomographic scan of the orbits to rule out any intraorbital pathology. Other less consistent findings in NXG that may be of limited value are a cryoglobulinaemia, a positive rheumatoid factor, depressed serum complement levels, and a reduced level of C1 esterase inhibitor. (If a review of the histology were carried out monoclonal antibody studies may identify the presence of T-helper cells within the granulomas which has been described in NXG.[5])

The increasing recognition of NXG as a specific clinicopathological entity with serious systemic associations means that this diagnosis must be considered in any case of a necrobiotic process affecting the periorbital region.

JONATHAN LUCK
*Department of Ophthalmology,
St James's University Hospital,
Leeds LS9 7TF*

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Reply

SIR,—I note with interest Mr Luck's suggestion that a diagnosis of necrobiotic xanthogranuloma should be included. This is a condition that I was not previously familiar with and I am grateful to him for drawing my attention to it.

T E LAVY
*88 Old Landsdowne Road,
Manchester M20 8WX*

BOOK REVIEWS

Graves' Ophthalmopathy: Current issues in endocrinology and metabolism. Eds Jack R Wall and Jacques How. Pp 196. £45.00. Blackwell: Oxford, 1991.

In 1989 the first international meeting devoted to thyroid eye disease was held in Montreal. In addition to endocrinologists and ophthalmologists there were immunologists, pathologists, radiotherapists, otolaryngologists and oculoplastic surgeons, geneticists, biochemists, and statisticians.

Despite such an array of expertise the first 78 pages, which are devoted to trying to expound the pathological processes, are far from conclusive. Autoantibodies to eye muscle can be demonstrated, but they show incomplete specificity, with some cross reactivity with diaphragm muscle and with thyroid antigens. Connective tissue antibodies and cell mediated immunity are also considered. Wall proposes a working hypothesis that Graves' ophthalmopathy follows the reaction of a primarily thyroid-directed cytotoxic antibody with an antigen present on the surface of the eye muscle membrane. Studies of T-lymphocyte reactivity to retrobulbar antigens is emerging as one of the key areas. However, the very protracted natural history of the condition and the problem of unilaterality of the proptosis in many patients are questions that will have to be answered by any proposed pathogenic mechanism.

The remaining 109 pages cover the problems of clinical management. Unfortunately there is still no universally agreed scheme to describe the various forms and levels of involvement of the eye and orbit in this condition. There is a useful chapter on the structure and mode of action of cyclosporin, but another chapter is given over to plasmapheresis, though most workers have abandoned this as a mode of treatment.

The long term follow-up of patients treated by orbital radiotherapy at Stanford under the direction of the late J P Kriss confirms the value of 2000 cGy of megavoltage irradiation in fractionated doses over a two-week period. Recent results from (West) Germany claim equal effectiveness for 'low dose radiotherapy' of only 6–8 cGy in total. Several of the workers comment on the active phase of Graves' ophthalmopathy and on the improved chances of therapeutic success during this period of the disease. Furthermore, many of them consider either combined or sequential therapy with prednisolone as the linchpin and cyclosporin or radiotherapy to be used additionally.

Finally, surgery is discussed and the various routes for orbital decompression, usually two or sometimes three wall decompression, are described. Surprisingly there is no detailed discussion of what is probably the most common surgical procedure in Graves' ophthalmopathy, namely ocular muscle surgery for diplopia. Eyelid operations, to reduce retraction of either the upper or the lower eyelids, are described.

Just as the combined activity of endocrinologist and ophthalmologist gives best treatment for the patient, so a meeting of this type which includes immunologists and biochemists should ensure that at least the correct questions are being asked with regard to the aetiology of this enigmatic condition. The inevitable unevenness of the contributions must not be allowed to detract from the value and purpose of a meeting on such an important topic. Each chapter is well referenced and provides continuing stimulation for all types of workers who are seeking solutions in this fascinating condition.

PETER FELLS

History of Ophthalmology 3: Sub auspiciis Academiae Ophthalmologicae Internationalis. Eds H E Henkes, Cl Zrenner. Pp 150. £61. Kluwer: Dordrecht, Netherlands, 1990.

Ophthalmology sadly lacks any magisterial record of its history. This is a subject of wide (and increasing) general interest, but the problems of amalgamating, interrelating, and balancing are enormous. The field is vast enough, but the real difficulties arise as we approach the last century when the narrative becomes so complex that it must fragment into subspecialties and take account of national schools (with constant proliferation in both fields); this almost inevitably calls for multiple authorship, leading to overlap, gaps, and imbalance in style and presentation. So one easily finishes up with an indigestible hotchpotch, punctuated by tedious catalogues of names.

In many respects the greatest mine of information on our earlier history is still to be found in Casey Wood's *American Encyclopedia of Ophthalmology* (1917), which is interspersed with extensive historical sections compiled by Shastid, albeit in a rather ruminative, avuncular style. Then in 1933 came a concise but very readable *Short History of Ophthalmology* by Sorsby, and thereafter, in 1962, a rather more turgid paperback in the 'Clio Medica' series by Chance; the latest is a rather ponderous survey by Gorin (1982), which was largely regimented in terms of the different national institutions.

When the International Academy of Ophthalmology was founded by François in 1960 (as the academic counterpart of the International Council of Ophthalmology), one of its first aims lay in preparing a comprehensive and authoritative history of our specialty. One volume was planned to cover its evolution up till 1850, then one covering each subspecialty in the years that followed, and a final volume dealing with each national school, along with short biographies of the more distinguished participants. When this plan foundered, Blodi (then our President) undertook to translate the historical sections of Hirschberg's monumental ten-volume series which covered the period until 1918, and of this eleven handsome volumes in English are now available. Meanwhile a surrogate was proposed in a different form by Henkes, who arranged for the Documenta Ophthalmica to relegate one of its monthly volumes each year to our International Academy for a collection of historical essays which he would edit. The first emerged in 1988, and this latest, third volume, certainly sustains the high standard of its predecessors, covering an agreeable diversity of aspects (art, literature, biography, and philosophy) punctuating the long history of our specialty.

P D TREVOR-ROPER

Oncology of the Eye and Adnexa: Atlas of Clinical Pathology. By A Brini, P Dhermy, J Sahel. Pp 154. £110.00. Kluwer: Dordrecht, The Netherlands, 1990.

This book is one of a series of monographs in ophthalmology published by Kluwer. It runs to 154 pages, half of which are devoted to a series of some 380 illustrations, the vast majority in excellent colour. The illustrations depict the clinical and histological appearance of most of the tumours of the eye and adnexa likely to be met by a practising ophthalmologist. The subject matter is dealt with from the eyelids and conjunctiva, through tumours of the orbit and orbitopalpebral tissues, to intraocular tumours involving the uvea, and finally the retina and optic disc. The text itself is unusual in that it is trilingual, each page of text being arranged in three columns: the first in English, the second in French, and the third in German. Each page of text is faced by a page on which are arranged six illustrations occupying the upper half of the page, while the lower half is devoted to useful and informative captions, again in the three languages arranged similarly in three vertical columns.

The authors' main aim spelt out in the introduction is the production of a practical reference manual for the practising ophthalmologist giving rapid access to the clinical and pathological features of tumours of the eye and adnexa and in this they succeed admirably. They have successfully married the clinical and pathological features of the tumours described both in the text and in the illustrations and it is likely that the book will, as the authors hope, be of value to pathologists and dermatologists in addition to ophthalmologists.

Criticisms are relatively minor, perhaps the most important being the small size of the illustrations necessitated by the need to compress a large number of illustrations into small bulk and the need to accommodate a trilingual text. Most of the clinical pictures are of good quality and reproduction of the histological sections is also good although at times a larger format or higher magnification would have been helpful if details described in the text were to be easily identified in the pictures.

The English text is a little stilted and there are a fair number of grammatical errors. These however do not compromise the meaning of the text nor do they detract greatly from the value of the book.

Because this is basically an atlas the text is greatly compressed. Nevertheless most of the points of importance in relation to each of the tumours described and illustrated are adequately made. No attempt however is made to deal with any of the basic aspects of oncology (except rather superficially in the case of retinoblastoma) and, although an indication of prognosis is given in each case, treatment is not discussed. There is a short appendix giving guidance to the ophthalmologist on how best to prepare an eye or a biopsy for pathological examination and also some guidance for the pathologist on how best to process an enucleated eye for satisfactory histological examination. Finally the book details the staining methods used for the illustrated histological preparations.

Within the rather restricted aims set by the authors this book successfully and succinctly presents a great deal of information about the clinical appearances and pathological features of most of the ophthalmic tumours likely to be met in clinical practice. The book would form an excellent revision text for those preparing for examinations in either ophthalmology or pathology and it can be recommended to both ophthalmologists and pathologists as a rapid source of information on ocular and adnexal tumours.

The large number of excellent colour illustrations is reflected in the price.

W S FOULDS

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NOTES

American Academy of Optometry

The annual meeting of the American Academy of Optometry will be held on 12–14 December 1992 at the Buena Vista Palace in Orlando, Florida, USA. The deadline for the submission of abstracts in printed form is 17 August and by electronic mail 24 August. Further details: Program Committee, American Academy of Optometry, 4330 East-West Highway, Suite 1117, Bethesda, MD 20814, USA.

American Academy of Ophthalmology

A 10 year cumulative index is now available for the publication *Focal points: clinical modules for ophthalmologists*, produced by the American Academy of Ophthalmologists. The following modules are being prepared for this tenth anniversary year: Corneal complications of contact lenses; Radial keratotomy for myopia; Periorbital animal bites; Cyclodestructive procedures for glaucoma; and Infectious keratitis. An annual subscription is $85 for academy members and $110 for non-members. Further details from: Customer Service, American Academy of Ophthalmology, PO Box 7424, San Francisco, CA 94120–7424, USA. (Tel: (415) 561–8540.)

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Editorial

Heavy liquids for postoperative tamponade

Heavy liquids (characterised by a high specific gravity relative to aqueous or subretinal fluid) have gained wide acceptance as intraoperative tools which aid and simplify vitreoretinal surgical manoevres in a variety of settings. These include anterior retinal breaks, giant retinal tears,[1] proliferative vitreoretinopathy,[2] and dislocated lenses or lens implants. Blinder and colleagues in this issue report on the use of heavy liquids for postoperative tamponade.

Postoperative tamponade agents such as air, gas, silicone oil, and, now, heavy perfluorocarbon liquids (PFCL) are used in vitreoretinal surgery because of their high interfacial surface tension in water, albeit a slightly lower surface tension in biological fluids. This means that they will obstruct fluid transfer through retinal breaks when in apposition to the break, thus maintaining retinal apposition until a secure laser or cryopexy scar is formed. A tamponade agent will achieve internal closure of a break if its volume is sufficient, the contact angle is favourable, and the eye is appropriately directed with respect to location of the retinal break and the relative density of the tamponade agent used. The unusual postoperative 'postures' required of vitreoretinal patients regularly provoke amusement, surprise, and sympathy while serving to emphasise the importance retinal surgeons place on maintaining break closure.

The contact angle and relative weight or buoyancy of a tamponade agent dictate how much of a given volume will be in contact with the retinal surface; the contact angle of silicone oil (40°) is similar to that of C_3F_8 gas (37°) but the contact area of a 1 ml gas bubble is much greater (205 mm^2) than a silicone oil bubble of the same volume (68 mm^2) because of the greater buoyancy of gas.[3] PFCLs have a much higher contact angle (105° for 1,3-dimethylcyclohexane) and this, together with the greater density disparity relative to water, results in a very high contact area (262 mm^2 for a 1 ml bubble).[3] PFCLs are therefore able to provide extremely effective tamponade especially for retinal breaks in the lower half of the retina (assuming an upright patient).

These comparisons imply that silicone oil, with its modest specific gravity disparity (0·95) and, therefore, low contact area should be a substandard tamponade agent, and yet it is clinically effective. Furthermore, the great majority of retinal breaks in mobile retinas can be closed with any internal tamponade agent. Why then have Blinder and colleagues reported the use of a high specific gravity tamponade? There is little doubt that postoperative PFCL tamponade will be of value in selected patients who are unable, or unwilling, to comply with post-surgical posturing; patients with inferior breaks are, however, relatively uncommon.

Whether PFCLs will prove more effective than current agents in the setting of proliferative vitreoretinopathy (PVR) will determine their real value.

Despite similar surface tension effects in aqueous (40–50 ergs/cm^2), PFCLs appear to be more effective than silicone oil in resisting tractional retinal detachment in experimental models of PVR.[4] This intriguing finding implies that the transretinal force exerted by the tamponade agent (0·06 g upward force for a 1·0 ml silicone oil bubble compared with 0·25 g downward force for a 0·3 ml PFC bubble) must be a significant factor limiting retinal detachment in the setting of experimental retinal traction. If such an effect can also be demonstrated in the clinical setting, it could prove highly beneficial in the management of PVR.

While a low viscosity is advantageous when using an intraoperative tamponade fluid since it aids injection, manipulation, and removal, this feature contributes to the complications associated with postoperative tamponade. Low viscosity is associated with an exaggerated tendency to emulsification, and also contributes to displacement of the fluid into the anterior chamber. If heavy liquids are to have a place in the postoperative management of retinal detachments, therefore, it is possible that the ideal characteristics of a postoperative heavy liquid could be quite different from those of an intraoperative heavy liquid. The high density and low viscosity of PFCLs mean that the fluid can be readily displaced within the eye. Though the surface tension is high, this cannot prevent a large mass of fluid passing through large 'holes' including the pupil (thus filling the anterior chamber). While this occurred in only one of Blinder's six aphakic eyes, Sparrow *et al*[4] have observed that aphakic eyes are highly susceptible to displacement of vitreous cavity PFC into the anterior chamber, which can result in severe endothelial cell damage.[5] Since the greatest use of postoperative PFCLs might be expected in eyes with PVR, almost all of which are aphakic, this could represent a major limitation.

The finding that silicone oil may absorb retinol, which is lipid soluble, from the retina,[6] implies that long term oil-retina contact may be undesirable. The question of the solubility of retinal metabolites in PFCLs is also of great interest, therefore, in relation to prolonged postoperative heavy liquid tamponade. Although PFCLs are not lipid or water miscible, they can dissolve large volumes of gases and selected low molecular weight molecules, so these properties could be expected to affect local retinal metabolism and function.

In addition to metabolite absorption from the retina, molecular exchange can occur between the tamponade agent and the retina and preretinal fluid. Impurities in intraocular tamponade agents are associated with cellular chemoattraction and reproliferation, thus emphasising the importance of achieving high degrees of purity for PFCLs.[7] Recently the concept of chemical exchange between intravitreal compartments has been extended to the use of postoperative tamponade agents such as silicone oil as depots for the gradual release of antiproliferative drugs such as 5-fluorouracil[8] and retinoic acid.[9] Since intravitreal depot drug release may become an important part of our management of PVR in the future, the question arises: which antiproliferative drugs could conceivably be delivered to proliferating cells from a PFC depot? Perfluorocarbons, such as the perfluorophenanthrene used by Blinder's group, are totally immiscible with water, and largely oleophobic. This presents a major impediment to storage of biologically active drugs, which must have some degree of water or lipid solubility in order to achieve cell entry. Highly perfluorinated drugs are soluble in PFCs,[10] and an antiproliferative drug with these properties could be useful. However, on theoretic grounds, none of the antiproliferatives currently under study (daunorubicin, retinoic acid, sodium butyrate,[11] and fluorouracil derivatives) are likely to be highly soluble in PFC liquids, though this question requires further study. Since PVR is the major problem currently facing vitreoretinal surgeons, this could be an important limiting factor. One solution would be to combine a PFC bubble with a low specific gravity tamponade such as silicone oil, which could also act as a drug delivery medium while acting on the superior retina.

Considerations other than that of interfacial surface tension may thus govern our choice of intravitreal tamponade agent in the future. The effects of viscosity, specific gravity, retinal metabolic exchange, and possible drug depot functions may assume greater importance in those settings where prolonged postoperative tamponade is required. As a result it is likely that in some situations single agent tamponade may no longer be the rule. Combined silicone oil and PFCL tamponade reduce the rate of retinal detachment in experimental PVR,[4] as well as reducing emulsification and displacement of heavy liquid into the anterior chamber.

The advent of postoperative heavy liquid tamponade raises major new questions for vitreoretinal surgeons. Careful prospective studies should illuminate those clinical settings in which these new properties can be used to greatest advantage.

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ORIGINAL ARTICLES

The electro-oculogram in central retinal vein occlusion

D Papakostopoulos, PA Bloom, RHB Grey, J C Dean Hart

Abstract

As part of a prospective masked study, the electro-oculogram (EOG) was recorded from 28 patients within 48 days of developing central retinal vein occlusion (CRVO). The EOG light peak/dark trough ratio (Lp/Dt)×100 was significantly lower in the affected than in the unaffected eyes of patients ($p<0.001$), and abnormally low in absolute terms in 20 patients (71%). All unaffected fellow eyes had a normal EOG ratio. The mean Lp amplitude of affected eyes was significantly smaller than that of unaffected eyes ($p<0.001$), whereas the differences in mean Dt amplitudes between affected and unaffected eyes were not statistically significant. The Lp amplitude in the affected eye was 48% or less of that in the unaffected eye in the eight patients (29%) who developed rubeosis iridis during the 9 month follow-up, and in six others. No patient whose Lp amplitude in the affected eye was greater than 48% of that in the unaffected eye, developed rubeosis. It is concluded that the Lp amplitude is abnormal in patients with acute CRVO. The degree of this abnormality bears a relation to the development of rubeosis, which might prove a useful indicator of whether to institute or withhold panretinal photocoagulation.

(*Br J Ophthalmol* 1992; 76: 515–519)

Central retinal vein occlusion (CVRO) is a common cause of visual loss and may lead to rubeosis iridis and to potentially painful neovascular glaucoma. The risk of this complication may be reduced by panretinal photocoagulation following the early identification of high risk cases. Attempts have been made to predict rubeosis using various parameters but no method of prediction has gained wide acceptance in clinical practice.

Electrophysiological investigations in cases of CRVO have largely been concerned with changes in the electroretinogram.[1-7] There are only a few contradictory references to electro-oculogram (EOG) changes in CRVO.[1 8-10] In his original description of the EOG, Arden described a case of CRVO in which the EOG was severely affected.[8] Ashworth reported four cases of CRVO, in all of which the EOG was affected to varying degrees.[9] Babel reported one case whose EOG was unaffected.[1] It was therefore decided to investigate, prospectively, the EOG changes in acute CRVO.

Patients and methods

PATIENT SELECTION

Patients found to be suffering from acute CRVO on presentation to the accident and emergency department of Bristol Eye Hospital, were referred to a special clinic for assessment. All patients presented within 3 weeks of noticing a visual disturbance.

The patients had a detailed clinical examination within 2 weeks of presentation. Patients then underwent electro-oculography within 10 days of clinical examination, and at least 7 days after instillation of any topical medication. This ensured that the effect of pupil dilatation did not influence the results.

The electrodiagnostic investigator (DP) was not provided with clinical details except that patients being assessed had recently suffered a CRVO in one or other eye. Patients were followed up clinically once a month by the same investigator (PB) for 9 months. The EOG results were only analysed after this period of follow-up. If rubeosis was noted during follow-up, panretinal photocoagulation (PRP) was performed.

A total of 28 patients (17 male, 11 female; age range 48–85 years, mean 67 years, SD 9 years) were included in the study. Two patients who were initially included, declined to undergo the tests. Eight patients developed rubeosis iridis.

CLINICAL EXAMINATION

At the initial clinic visit and at each subsequent clinical assessment, corrected visual acuity was measured and careful slit-lamp examination performed, including gonioscopy, to detect the earliest sign of rubeosis iridis. This was considered to be the appearance of rubeotic vessels at the pupil margin or in the iridocorneal angle. In this examination, consideration was given to the fact that early rubeosis may be difficult to see in patients with dark irides. If rubeosis was suspected the patient was reviewed weekly until progression of the subtle signs enabled a definite diagnosis to be made.

Pupil sizes were measured under room illumination, but in no patient was anisocoria more than 1 mm. Fundal examination was performed after pupillary dilatation. A clinical diagnosis of CRVO was made by identification of the typical fundal signs of CRVO, namely dilated and tortuous retinal veins, retinal haemorrhages at the posterior pole extending towards the mid-

Bristol Eye Hospital, Lower Maudlin Street, Bristol BS1 2LX
D Papakostopoulos
P A Bloom
R H B Grey
J C Dean Hart

Correspondence to:
Philip A Bloom, Moorfields Eye Hospital, City Road, London EC1V 2PD.

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periphery in all four quadrants, and unilateral disc swelling in association with a reduction of visual acuity.

INCLUSION AND EXCLUSION CRITERIA

Only patients with a definite clinical diagnosis of acute CRVO and who underwent clinical assessment and electro-oculography within 60 days of the onset of visual symptoms were included in the results of the study.

Criteria for exclusion from the study were previous significant ophthalmic disease in either eye, previous ophthalmic surgery, the use of topical or systemic medications, and previous photocoagulation.

EOG MEASUREMENT

The technique employed for recording the EOG was based on that of Arden *et al*[8] and has been described previously.[11] In summary the subject was seated 1·5 m in front of the centre of a panel, upon which were mounted eight neon tube bulbs. Two small red bulbs served as fixation points and were positioned either side of the centre of the screen to subtend an angle of 30° at the patient. Chlorided silver electrodes were attached to the bridge of the nose and to each outer canthus, and the signals were amplified by an EEG machine.

The subject was instructed to perform fast eye movements from one fixation point to the other, at a rate of one per second; a sequence of eight of these movements was performed and recorded every 2 minutes. Six such sequences were performed in darkness after a 20 minute period of light adaptation. Then with the room and screen illuminated another six sequences were recorded, again each separated by 2 minutes. The mean EOG amplitude for each sequence was calculated. Therefore for each subject, 12 values were obtained from the left and 12 from the right eye in each session, from which were determined the minimum EOG amplitude in the dark (dark trough, Dt) and the maximum EOG value during light (light peak, Lp). The whole procedure lasted approximately 35 minutes. The head position, eye movements, and pupil diameter of the subject were monitored using an infrared closed circuit TV camera.

Table 1 EOG Lp/Dt ratios of all patients, ranked in ascending order according to the ratio in the affected eye

Subject index	*EOG ratio in affected eye (%)*	*EOG ratio in unaffected eye (%)*
10*	86	255
24	95	238
1	100	301
2*	100	346
6*	100	316
4	100	249
20	100	262
27*	100	300
17*	100	243
26*	100	250
13	100	275
11	100	301
16*	100	227
7	110	250
15	117	264
5	120	264
25*	129	274
9	145	223
8	146	219
19	148	205
23	177	216
12	188	250
21	189	190
3	200	220
14	208	244
28	227	241
18	300	424
22	382	363
Mean	145	265
SD	68	50

* Rubeosis iridis

All results were analysed using two-tailed paired or unpaired *t* tests, as appropriate.

Results

Twenty eight patients were included in the study. Patients underwent electro-oculography between 14 and 48 days from onset of CRVO (mean 32 (SD 8) days). Eight patients (29%) developed rubeosis iridis during a 9 month

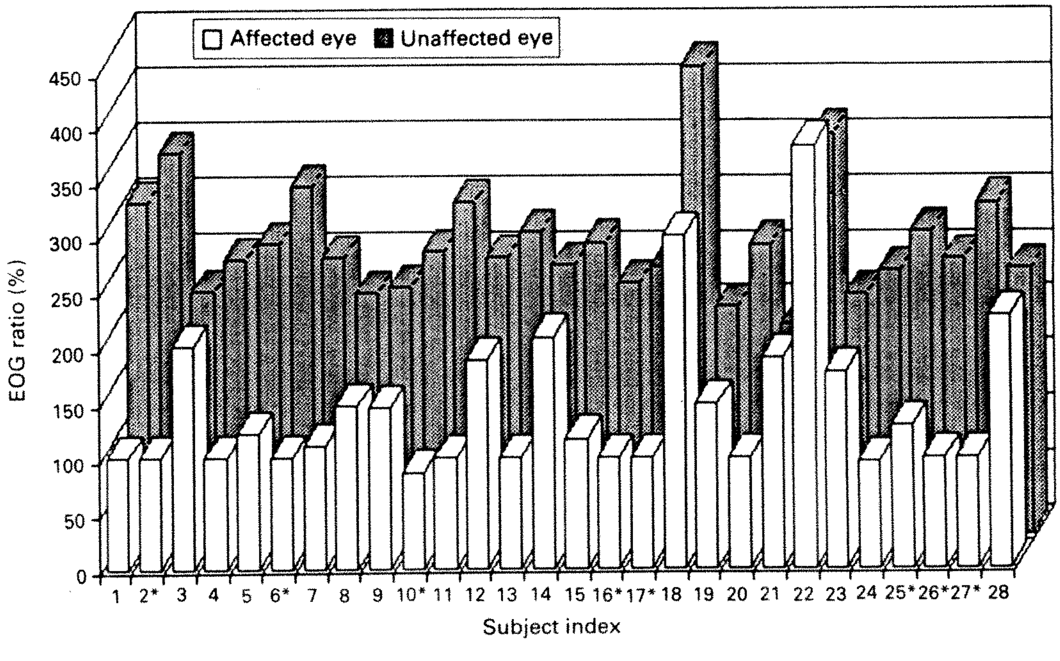

Figure 1 The EOG Lp/Dt ratio in the affected and unaffected eyes of patients with acute CRVO (: rubeosis iridis).*

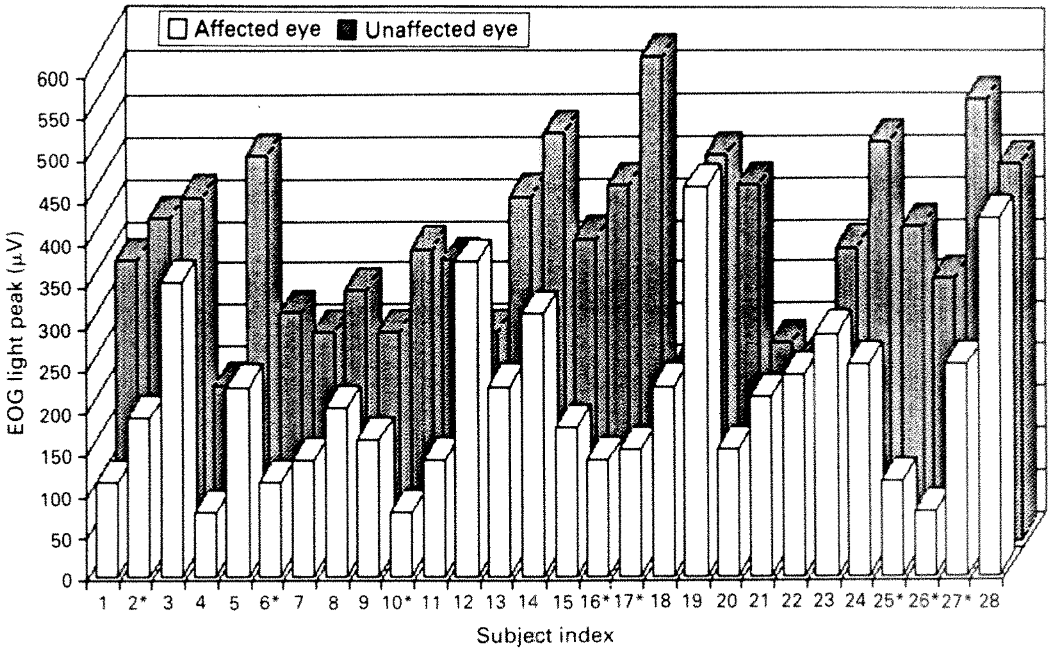

Figure 2 The EOG Lp amplitude in the affected and unaffected eyes of patients with acute CRVO (: rubeosis iridis).*

follow-up period. The rubeosis began at the pupil margin in six of these eight patients, on the trabecular meshwork alone in one patient, and at both of these sites in the remaining patient.

The EOG ratio (Lp/Dt)×100 was smaller in the affected than in the unaffected eye of all but one patient (Fig 1). The range of the EOG ratios for the unaffected eyes was 190–424%, and 86–382% for the affected eyes (Table 1). In 20 patients (71%) the EOG ratio of the affected eye was less than 170%. An EOG Lp/Dt ratio of less than 170% is considered abnormal in our laboratory and in others.[2 11]

The mean value of the EOG ratio of all affected eyes was 146% (SD 68%), and of all unaffected eyes 265% (SD 50%). This difference is statistically significant ($p<0.001$). Table 1 shows the EOG ratios of all patients, ranked in ascending order by the affected eye to facilitate interpretation of the data.

The EOG Lp/Dt ratio can be affected by changes in either the Lp or Dt. The mean Lp amplitude of affected eyes (208 (SD 101) μV) was significantly smaller ($p<0.001$) than that of unaffected eyes (361 (SD 100) μV). This is illustrated in Figures 2 and 3 (particularly in time intervals 11 and 12). The difference between the mean Dt amplitude of affected eyes (150 (SD 60) μV) and unaffected eyes (142 (SD 47) μV) was not statistically significant. This is shown in Figure 3 (time intervals 6 and 7).

To facilitate comparisons the amplitude of the Lp of the affected (A) eye was expresssed as a percentage ratio of that of the unaffected (NA) eye and the patients were ranked in ascending order according the value of this ratio (A/NA)×100 (Table 2). Fourteen patients had an A/NA ratio of less than 50%. It can be seen from

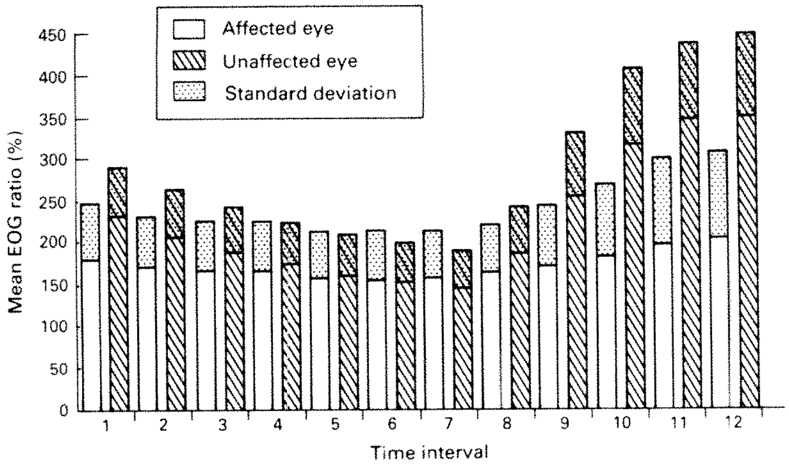

Figure 3 The mean and standard deviation of all patients' EOG Lp/Dt ratios in each time interval of 2 minutes. Time intervals 1–6 were during dark and time intervals 7–12 were during light (n=28). It can be seen that the largest differences between affected and unaffected eyes occurred in the light (time intervals 11 and 12), and that at the end of the dark period (time interval 6) there were no differences between affected and unaffected eyes.

Table 2 LP amplitude (μV) of the affected (A) eye, expressed as percentage ratio of that of the unaffected (NA) eye. The patients are ranked in ascending order according to the value of this ratio 100 A/NA

Subject index	*EOG light peak in affected eye (A)*	*EOG light peak in unaffected eye (NA)*	*100 A/NA (%)*
10*	75	350	21
26*	75	312	24
17*	150	575	26
25*	112	375	30
16*	137	425	32
1	112	337	33
20	150	425	35
4	75	187	40
11	137	337	41
6*	112	275	41
27*	250	525	48
2*	187	387	48
15	175	362	48
5	225	462	49
24	250	475	53
13	225	412	55
7	137	250	55
14	312	487	64
9	162	250	65
8	200	300	67
23	287	350	82
3	350	412	85
21	212	237	89
28	425	450	94
19	462	462	100
22	237	225	105
18	225	212	106
12	375	250	150
Mean	208	361	
SD	101	100	

*Rubeosis iridis

Table 2 that all the eight patients who developed rubeosis iridis had an A/NA Lp ratio at presentation of 48% or less, and that no patient developed rubeosis whose A/NA Lp ratio at presentation was greater than 48%.

When patients with EOG Lp/Dt ratios above 170% were compared with those below 170%, there were no statistically significant differences in age, sex, or time from CRVO onset to EOG testing, between the two groups. Nor were there any statistically significant differences in these parameters between those patients with an A/NA Lp ratio above 48% and those with an A/NA Lp ratio of 48% or below.

Discussion

The results presented above show that in this prospective masked study the EOG Lp/Dt ratio is significantly reduced in the affected eyes of a group of patients with CRVO compared with the unaffected fellow eyes. Further to this group effect the EOG Lp/Dt ratio was in the range that is considered abnormal (less than 170%[1 2 8 11]) in the affected eye of 71% of these 28 individuals, whereas that in the fellow eye was 190% or more in all cases. The EOG Lp/Dt ratio in the affected eye of the remaining 29% of cases was within the normal range in absolute terms, with minimal differences between the two eyes. This may partly explain the negative findings of previous studies.[1 9] It has been suggested that recovery of initial EOG abnormalities in patients with CRVO may occur later in the natural history of the condition.[9 10] This too may explain the differences between the findings of this study and of others, for EOG measurements in this study were made at the onset of the condition.

The results show that the effects upon the EOG Lp/Dt ratio are largely due to a reduction in the amplitude of the light peak (Lp). To explain why CRVO should lead to a reduction in the amplitude of the Lp, the nature of the damage in CRVO should be considered in the light of current understanding of the processes underlying the development of the Lp.

The blood circulation of the inner retinal layers is by the central retinal artery and the central retinal vein.[12] The RPE, photoreceptors, and outer layers of the neuroretina are supplied by diffusion of oxygen from the choroidal circulation. The watershed between the two circulations is at the level of the bipolar cell dendrites, at the junction of the outer and middle thirds of the outer plexiform layer of the retina.[12 13] It is thought that CRVO leads to ischaemia of the inner retina owing to reduced perfusion consequent upon the outflow obstruction.[14]

The dependence of the Lp on a normally functioning neuroretina has been repeatedly proposed from experimental evidence,[15–19] and it has been suggested that structures within the neuroretina contribute to the development of the Lp, in addition to the photoreceptor/RPE complex. The Dt is believed to reflect the function of the RPE alone,[8] and so impaired retinal circulation would not be expected to produce defects in the Dt. Moreover, it has been noted in animal experiments that the EOG Dt is not affected by central retinal artery occlusion.[20]

That CRVO has been found to affect the Lp implies either involvement of inner elements of the neuroretina in the development of the Lp, or extension of the damage consequent upon CRVO to involve the RPE and other structures supplied by the choroidal circulation.

Modulation of the standing potential of the RPE is thought to be initiated by chemical factors, but there is debate whether these modulating chemicals are released from the photoreceptors[2] (supplied by the choroidal circulation) or from more internal retinal structures[15–20] (supplied by the retinal circulation).

When the amplitude of the Lp in the affected (A) and unaffected (NA) eyes were compared by expressing these values as a percentage ratio A/NA, it was found that the ratio ranged from 21–150% (Table 2). When these values were ranked in ascending order it was observed that only those patients whose ratio was 48% or less developed rubeosis. In other words, if the amplitude of the Lp of the EOG in the affected eye is less than half of that of the unaffected eye, then there is an increased probability that rubeosis will develop. When the EOG Lp/Dt ratio was similarly ranked, it was not as effective as the A/NA ratio in predicting rubeosis (Table 1). This may be due to an effect upon the EOG of the across-subject variability in the Dt ratio.

It is important to determine that in those patients with an A/NA ratio of 48% or below, or those with an EOG Lp/Dt ratio below 170%, the observed increased incidence of rubeosis was not due to other factors. These populations were therefore examined with respect to age, sex and time between CRVO onset and EOG testing. They were compared with their fellow populations of patients whose A/NA ratio was above 48%, and with those whose EOG Lp/Dt ratios were above 170%, respectively. No statistically significant differences were found between the two sets of populations.

Whatever the case, the results appear to be clinically relevant because the degree of EOG abnormality bears some relation to the development of rubeosis iridis. (Rubeosis may also be caused by other ischaemic conditions such as diabetes mellitus and carotid insufficiency.) CRVO is a common cause of loss of vision and may lead to rubeosis (in 29% of patients in this series), and to potentially painful neovascular glaucoma in 8–25% of patients.[21] The risk of these complications, which are more common in ischaemic CRVO, may be significantly reduced by adequate PRP.[21–24] However, because of the side effects of PRP (including constriction of the visual fields in these patients, who often have poor central vision), this treatment is only indicated in ischaemic cases where the risk of rubeosis is high. Despite this, it has been reported that up to 80% of patients with CRVO are treated with PRP.[24] A major clinical problem in CRVO is the early identification of high risk cases. Attempts have been made to predict rubeosis using electroretinographic parameters[3–7] but not, as far as we have been able to ascertain, using the EOG. Because no single method of prediction is ideal none has gained wide acceptance in clinical practice.

In this study no patient whose A/NA EOG Lp ratio was more than 48% developed rubeosis. Of the 14 patients whose A/NA Lp ratio was less than 50%, eight developed rubeosis, five of whom were the patients with the smallest of all A/NA Lp ratios. However six of these 14 did not develop rubeosis in spite of a low ratio. The data does not explain in what way these six patients are different.

Further work is required to investigate these aspects and the variation of the EOG with time, for the results above represent readings taken at only one point in the natural history of the condition. However the results do show that no patients developed rubeosis if their A/NA ratio was greater than 50%, which may develop into an important finding in the clinical management of CRVO.

Conclusion

It has been shown that the EOG is affected in many patients with acute CRVO, and that this is due to an effect on the EOG light peak. This may suggest that retinal control of the EOG occurs in the inner retina but a toxic effect cannot be ruled out. The results have been shown to have clinical relevance because patients with the most severely affected EOG most commonly develop rubeosis. Further investigation into EOG changes in the pathological situation of CRVO may aid our understanding of normal and abnormal retinal function.

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Controlled study of ocular morbidity in school children born preterm

F G McGinnity, J H Bryars

Abstract

Two hundred children who were of very low birth weight (VLBW) (1500 g or less) and 193 controls who were of normal birth weight (NBW) were examined at approximately 9 years of age. Binocular visual acuity of 6/6 or better was noted in 178 (89·5%) VLBW children and 189 (97·9%) NBW children. Visual morbidity was significantly higher among VLBW children. Strabismus was present in 38 (19%), cicatricial retinopathy of prematurity in 13 (6·5%), and optic atrophy in six (3%) children in this group. Children who were VLBW were also more myopic than the NBW controls. Optic atrophy was frequently associated with cerebral dysfunction. Regular assessments to identify ocular abnormalities in children who were VLBW are recommended.

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Advances in obstetric and neonatal care have significantly increased the survival of very low birth weight (VLBW) infants and in one area survival rates for these babies have improved from 35% to 72% between 1968–1972 and 1979–1983.[1] There has been considerable concern that the prevalence of neurosensory disorders among the survivors may also have increased[2] and recent reports have shown a high incidence of ocular problems among children who were born preterm.[3 4] However these studies were uncontrolled and performed on preschool children and do not give an accurate assessment of the visual performance of these children in relation to their contemporaries. The aim of this study was to compare the ophthalmological outcome of a group of children of school age who were VLBW with a control group of children who were full term infants.

Patients and methods

The study group comprised those infants of VLBW (1500 g or less), admitted to the neonatal intensive care unit (NICU) at the Royal Maternity Hospital, Belfast during the years 1978 to 1981, who survived. During this period, 381 babies were admitted to this NICU and 243 (64%) survived until discharge. A further five children died between discharge and the commencement of this study. Two hundred (84%) of the 238 children were available for a follow-up ophthalmological examination at a mean age of 9·1 years (range 7·2–11·3 years). Of the 200 children examined 68 (34%) had been transferred from another hospital during the neonatal period. Twenty children were extremely low birth weight (ELBW) – that is, weighing less than 1000 g and 49 were assessed as less than 28 weeks' gestation.

A control group of normal birth weight (NBW) children, who had weighed more than 2500 g at birth, was chosen. Each child in the VLBW group was matched with a child in the control group on sex of the child, date of birth, and social class. The distinguishing variables between the two groups were gestational age (preterm versus full term) and birth weight (less than 1500 g versus more than 2500 g). In total the control group comprised 193 subjects. The mean age of the VLBW children at examination was 9·10 years (SD=1·05) and that of the NBW children was 8·82 years (SD=1·02). The sex distribution of children in the two groups was roughly equal with 83 males and 117 females in the VLBW group. Using the registrar general's classification, there was no significant difference between the VLBW and NBW groups when social class was considered.

All eye examinations were performed by a single observer (FGMG). Where possible monocular and binocular visual acuities were tested using a Snellen chart. Visual acuity was classified as good (6/6 or better), intermediate (6/9 to 6/12), or poor (6/18 or worse). Three children who were unable to comprehend the Snellen test were assessed with graded white balls from the Stycar vision testing set.[5] Binocular reading vision was assessed using near test types. A comprehensive assessment of ocular motility was performed on each child. Details of previous orthoptic treatment were obtained from hospital records.

A cycloplegic refraction was carried out following the instillation of cyclopentolate 1% in both eyes. The spherical equivalent for each eye was calculated as the mean of the refraction values in the two principal meridians. The spherical equivalent value for each child was calculated as the mean of the spherical equivalents for each eye. Anterior segment examination with a slit-lamp and funduscopy with a direct ophthalmoscope were performed in all children. The peripheral fundus was examined with an indirect ophthalmoscope after pupillary dilatation. Regressed retinopathy of prematurity (ROP) findings were documented according to the scheme devised by the International Committee for the Classification of the Late Stages of ROP.[6] Electrophysiology was performed, when necessary, to supplement the clinical examination.

Of the present cohort of 200 VLBW children, 191 had been examined by an ophthalmologist during the neonatal period. This included a fundal examination after pupillary dilatation. The presence of any ocular abnormality including ROP was noted at that time.

Data were analysed using the SPSS-X[7] statistical package on an IBM 3090 computer. Where a mean value was calculated the corres-

Department of Ophthalmology, Royal Victoria Hospital, Belfast
F G McGinnity
J H Bryars

Correspondence to:
F G McGinnity, Department of Ophthalmology, Eye and Ear Clinic, Royal Victoria Hospital, Belfast BT12 6BA.

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Table 1 Details of ophthalmic findings and mental retardation of VLBW children with poor binocular visual acuity

Case no	*Visual acuity*	*Ophthalmic findings*	*Mental handicap*	*Special schooling*
1	6/18	none	Down's syndrome	yes
2	6/18	nystagmus	none	no
3	6/24	myopia	none	no
4	6/24	albinism	none	no
5	6/60	ROP	none	yes
6	6/60	none	yes	yes
7	HM	optic atrophy	yes	yes
8	HM	optic atrophy	yes	yes
9	HM	optic atrophy	yes	yes
10	NPL	optic atrophy	yes	yes

HM=hand movements; NPL=no perception of light; ROP=retinopathy of prematurity.

ponding standard deviation (SD) was also noted. Where there was a significant difference the appropriate test and the significance level were cited. A test was considered not significant (NS) if it failed to reach the $p=0{\cdot}05$ level.

Results

VISUAL ACUITY

Binocular visual acuity was assessed in 199 of 200 VLBW children and all of the NBW children. Vision assessment was not possible in one child with Down's syndrome. Visual acuity was significantly poorer in the VLBW group ($\chi^2=13{\cdot}51$, DF=2, $p<0{\cdot}001$). Whereas 189 (97·9%) NBW children had 6/6 vision or better, 178 (89·5%) VLBW children had similar vision. No child in the NBW group had 6/18 or poorer vision compared with 10 (5·1%) in the VLBW group. Table 1 lists details of the 10 children in the VLBW group with poor vision including significant ophthalmic findings, associated mental retardation, and whether special schooling was needed.

All of the children in the NBW group achieved a reading vision of N5. Reading vision could only be tested in 189 (94·5%) children in the VLBW group because of poor cooperation. Of these only two children failed to read N5, one child reading N12, and the other N6. Overall, 188 (94%) of the VLBW group had a reading vision of N6 or better.

Best corrected monocular visual acuity was assessed in 190 children in the VLBW group and all children in the NBW group. Three children in the VLBW group were excluded because it was not possible to test each eye separately and a further seven children failed to attend wearing an appropriate spectacle correction. Whereas there were only four (1·1%) eyes with 6/18 or poorer vision in the NBW group, this compared with 25 (6·6%) eyes in the VLBW group. The different causes of poor monocular vision among the VLBW group are listed in Table 2. Miscellaneous causes of poor monocular vision found in four eyes, included one child who had nystagmus associated with hydrocephalus and another who had oculocutaneous albinism.

Table 2 Causes of poor monocular vision in 25 eyes of children who were very low birth weight

	No of eyes
Strabismic amblyopia	7
Optic atrophy	6
ROP	5
Myopia	3
Miscellaneous	4

OCULAR MOTILITY

A concomitant squint was noted in 38 (19%) of the VLBW group and in five (2·5%) of the NBW group and the difference is highly significant ($\chi^2=27{\cdot}14$, DF=1, $p<0{\cdot}001$). A further 10 children had a past history of squint, four of whom had been treated surgically while six responded to conservative treatment. There was no significant difference between the ratio of convergent to divergent squint in the VLBW group (3·4:1) and in the NBW group (2·5:1). Among the VLBW group, six children with birth weight of less than 1000 g had squint compared with 32 weighing more than 1000 g (NS).

Squint was associated with optic atrophy (five) and cicatricial ROP (six) in 11 children who were VLBW and with Goldenhar's syndrome in one NBW child. When these structural abnormalities of the visual pathways were excluded there still remained a significant difference in the frequency of squint between the VLBW group and the NBW group ($\chi^2=18{\cdot}97$, DF=1, $p<0{\cdot}001$). When a further four VLBW children with squint and cerebral palsy (see below) were excluded there were 23 (12·4%) children in the VLBW group with strabismus and this was still significantly different from the NBW group ($\chi^2=9{\cdot}93$, DF=1, $p<0{\cdot}01$).

Sixteen children in the VLBW group had cerebral palsy. Eight (50%) of these children were noted to have squint and this represented 21% of the VLBW group with squint. Three of these children were noted to have optic atrophy and a fourth had cicatricial ROP. Therefore only in four (25%) of these children could the squint be associated with cerebral palsy alone.

Extraocular movements were studied in all children. One child who was VLBW had bilateral Duane's syndrome while another had a unilateral Brown's syndrome. Both children were orthophoric in the primary position. Among the NBW group one child had nystagmus associated with Goldenhar's syndrome and another had limitation of adduction in one eye. Nystagmus was observed in nine children who were VLBW, five of whom had optic atrophy. Of the remainder one child had nystagmus associated with hydrocephalus, another oculocutaneous albinism and two had cicatricial ROP.

REFRACTION

A cycloplegic refraction was performed in all but one of the children who were VLBW and all eyes but one in the NBW group. The spherical equivalent value for each child was calculated and their distributions are presented in Figure 1. The mean spherical equivalent of children who were VLBW was 0·99 D (SD 2·47) and 1·67 D

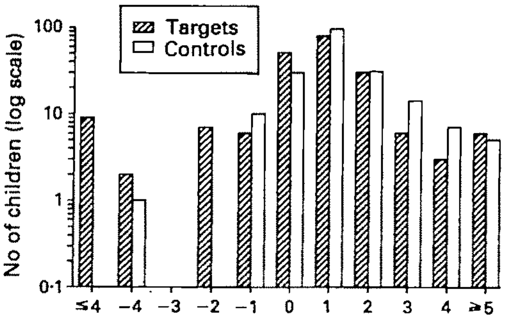

Figure 1 Spherical equivalent values (mean spherical equivalent of two eyes) of children who were of very low and normal birth weight.

(SD 1·31) for NBW children ($t=3\cdot37$, DF=390, $p<0\cdot001$). A minus spherical equivalent value was present in 24 (12·1%) children who were VLBW and in 11 (5·7%) NBW children ($\chi^2=4\cdot87$, DF=1, $p<0\cdot05$).

When children with regressed ROP were excluded from the analysis the mean spherical equivalent of the remaining children was 1·24 D (SD 2·13) and there still remained a significant difference betweem this VLBW group and the NBW group ($t=2\cdot40$, DF=373, $p<0\cdot05$). Although 17 (9·3%) children in this VLBW group without regressed ROP had a minus spherical equivalent value, this did not represent a statistically significant difference between this group and the NBW group.

ANTERIOR SEGMENT

Significant corneal pathology was noted in only one child in the NBW group who had bilateral limbal dermoids associated with Goldenhar's syndrome. One child in the VLBW group had cataracts diagnosed at 3 years of age. Subsequently he had bilateral lensectomy and at the time of examination visual acuity was 6/6 in his right eye and 6/12 in the left with aphakic correction. This child had hypocalcaemia but was neither asphyxiated or hypoglycaemic in the perinatal period. There was no family history of cataract. Bilateral temporal sector lens opacities were also noted in a child with cicatricial ROP.

Visually insignificant lens opacities were also observed. These included Mittendorf dots noted in six (nine eyes) children who were VLBW and eight (10 eyes) NBW children. Other minor changes including multiple dots and sutural lens opacities were noted in five (10 eyes) children in the VLBW group and three (five eyes) NBW controls.

OPTIC ATROPHY

Optic atrophy was noted in six (3%) children who were VLBW. Four of these children with bilateral optic atrophy were severely mentally retarded. Another child had unilateral optic atrophy associated with cicatricial ROP. Lastly a child who initially presented at age 4 with squint was noted to have nystagmus and bilateral optic atrophy at follow-up. Visual acuity was reduced to 6/24 in the right eye with absent pattern visual evoked response. Focal dilatation of the left lateral ventricle on computed tomographic scan was thought to be related to periventricular ischaemia in the perinatal period. There was a history of pre-eclamptic toxaemia during the pregnancy and the child required resuscitation at birth. He subsequently developed necrotising enterocolitis.

Of the six children with optic atrophy three had intraventricular haemorrhage (IVH) and four had seizures in the perinatal period. Children with optic atrophy were also more likely to have had bronchopulmonary dysplasia and hyponatraemia as neonates. However, this group was no more likely to have had fetal distress or poorer Apgar scores than children in the VLBW group without optic atrophy.

REGRESSED ROP

The typical 'dragged disc' of more severe posterior regressed ROP was noted in six (3%) children. Two of these children weighed less than 1000 g at birth. In two cases the disease was asymmetrical with only minimal changes and good vision in the fellow eye. Myopia was associated with posterior cicatricial ROP in all but one child with bilateral disease who had an uncorrected vision of 6/9 in the right eye and 6/12 in the left. Although one child was legally blind with the disease no case of total retinal detachment was found.

Indirect ophthalmoscopy was performed in 195 children who were VLBW and all of the children in the NBW group. Evidence of peripheral regressed ROP was noted in seven children who were VLBW. The commonest finding was temporal pigmentation. Vitreoretinal interface abnormalities, areas of avascular retina and lattice degeneration were also noted. Retinal tears in areas of lattice degeneration were present in two children, one of whom required cryopexy. Four of these seven children had significant associated ocular disease; two with myopia and two with squint. In total 13 (6·7%) of the VLBW children were noted to have evidence of regressed ROP. No similar retinal pathology was noted in the NBW children.

Only two children in this study were noted to have acute ROP in the perinatal period. One child developed cicatricial changes while in the other child the disease totally regressed. Another child who had avascular peripheries as a neonate was noted to have similar changes with associated pigmentation on follow-up.

Discussion

Two hundred (84%) surviving children, from one neonatal intensive care unit, were examined in this study. This is the most comprehensive controlled follow-up of VLBW children of school age undertaken within the British Isles in recent years. Ocular morbidity was high among the VLBW group and this may be due to bias in the sample since those children with known defects were more likely to attend for ophthalmic examination. However, of the 38 surviving children who did not have an eye examination, at least two were known to have strabismus and one had poor vision.

Binocular visual acuity of 6/18 or worse was recorded in 10 (5·1%) or 1:20 of the VLBW children. This contrasts with an incidence of visual handicap among children in Northern Ireland of less than 1:1000.[8] The frequency of vision worse than 6/18 is also higher than similar follow-up studies of preterm children where frequencies of 1%[9] and 2·4%[10] have been noted. Although a higher rate of poor vision among VLBW children has been reported recently,[4 11] Snellen equivalent were not quoted and therefore direct comparison is difficult. No previous follow-up studies of children born preterm have tested reading vision. It is encouraging, especially from the educational viewpoint, that 188 (94%) children in the VLBW group had reading vision of N6 or better.

A concomitant squint was detected in 38

(19%) children in the VLBW group and strabismic amblyopia was the commonest cause of poor monocular vision. In similar studies of low birth weight groups 19%[3] and 22·5%[12] of children have been noted to have strabismus. When children with associated ocular abnormalities and cerebral palsy were excluded there were 23 (12·4%) VLBW children with squint and this is well above the prevalence of 3·5% found in the general population.[13] McCormick *et al*[9] found 14% of children with squint in low birth weight groups when other ocular or cerebral diseases were excluded.

Six (30%) children who weighed less than 1000 g at birth had strabismus and in a recent study of 4 year old children who were ELBW,[14] 24% were noted to have strabismus. Although we have noted a relatively high frequency of squint among ELBW children, this did not represent an excess in this group and is in keeping with other reports showing no correlation between squint and birth weight.[10 15]

Nystagmus was noted in nine children in the VLBW group, the majority of whom had optic atrophy or cicatricial ROP. In previous follow-up studies[4 16] of low birth weight children where nystagmus has been noted it has invariably been associated with either ocular or cerebral disease.

Of the 16 children with cerebral palsy 50% were noted to have squint and this is in agreement with previous reports[17 18] of the frequency of strabismus associated with cerebral palsy. The contribution that children with cerebral palsy make to the overall frequency of squint among VLBW children depends on the frequency of cerebral palsy in that group. Whereas 8% of VLBW children in our study had cerebral palsy, and this accounted for 21% of the cases of strabismus, Keith and Kitchen[3] noted 13·5% of children with cerebral palsy accounting for 33·3% of their cases of squint.

Eyes of children in the VLBW group were significantly more myopic with a mean refraction of 0·99 D than those in the NBW group whose mean refraction was 1·67 D. The latter falls between the general population mean ocular refraction of 1·25 D for boys and 2·03 for girls at 9 years of age.[19] Fledelius[10] also noted a myopic shift among a premature group with an average refraction of 0·75 D compared to a mature group with a mean refraction of 1·53 D. Most other reports have concentrated on the percentage of eyes of children with a minus refraction and from 13% to 23%[10 12 20] of children born preterm have been found to have myopia. The frequency of myopia in our study was 12·1% and in keeping with these reports was significantly higher than the control group.

The relationship between myopia and cicatricial ROP is well established.[21 22] Some follow-up studies of preterm infants have also noted that myopia is uncommon when cases of ROP are excluded.[23 24] When we exclude eyes of children with regressed ROP from the VLBW group, there was still a significant difference in the mean spherical equivalent between this group and the control group. A minus spherical equivalent was present in 9·3% of eyes of children in the VLBW group without ROP and this compares with a recent report[12] where 10% of a similar group of children had myopia. In contrast, it has been suggested[25] that children born preterm with high refractive errors and ophthalmologically normal fundi, may in fact have had acute ROP in infancy that completely regressed. We have found that preterm children without clinical signs of regressed ROP tend towards myopia and it has been noted that this myopia is mainly corneal in nature.[10] Light may have an inhibitory effect on the growth of the eye[26] and the thermal deficit resulting from premature birth may also adversely affect corneal flattening[27] which normally proceeds rapidly in the final weeks of gestation.

One child who was VLBW was noted to have isolated cataracts. Although this child had hypocalcaemia in the perinatal period it is unlikely this was the underlying cause of the lens opacities. Hypocalcaemia occurred in up to 68% of neonates during this period and these cataracts were not diagnosed until 3 years of age. Another child was noted to have cataract associated with cicatricial ROP. The overall frequency of lens opacities of 1% is similar to other studies of children born preterm[10 28] and contrasts with a prevalence of visually significant cataract in children, in Northern Ireland, of 0·03%.[8] There was no difference in the frequency of visually insignificant lens opacities between the two groups in this study and it has been noted that such changes are so minor as to be considered physiological.[29]

Optic atrophy was noted in six (3%) VLBW children and this compares with recent reports where 2·7% of VLBW[3] and 4% of ELBW[14] infants were found to have optic atrophy. Perinatal hypoxia may precipitate optic atrophy but this is an uncommon sequel of this metabolic disturbance[30] and we have found that children with optic atrophy were no more likely to have had fetal distress or poorer Apgar scores than the remainder of the VLBW children. Three children with optic atrophy were noted to have clinically significant IVH in the neonatal period and another child had evidence of previous periventricular ischaemia. The latter had many of the risk factors associated with this condition including pre-eclamptic toxaemia and necrotising enterocolitis.[31] Damage to the optic radiation and visual cortex in the perinatal period from hypoxic/ischaemic lesions or IVH may have resulted in trans-synaptic degeneration and optic atrophy in these children. However, although trans-synaptic retrograde degeneration has been noted in the visual system of adolescent primates[32] no evidence exists at present for a similar mechanism in humans.

Significant cicatricial ROP was present in six (3%) of VLBW children rising to 10% for those weighing less than 1000 g. Although there were no children who had bilateral retinal detachments from ROP, one child was registered blind. Of these six children with posterior cicatricial ROP only one child was noted to have acute ROP in the neonatal period. As many of these neonates were seriously ill for a considerable time their initial ophthalmological assessment was often delayed and acute stages of the disease may have regressed by the time they were examined. Subtle changes of cicatricial disease may have been overlooked at these later examinations.

In recent studies 1%[33] and 10%[3] of VLBW children have been noted to have cicatricial ROP. Variations in the reported rates of this condition may be influenced by the survival rate of the most premature infants and up to 42% of children who were ELBW[34] have been found to have cicatricial ROP. In our study only 10% of children weighed less than 1000 g, contributing to a low overall frequency of cicatricial ROP. In contrast to the severe blinding cicatricial disease during the epidemic years,[35] cicatricial ROP during the period studied in this neonatal unit appears much less severe. Perhaps better control of oxygen therapy with blood and transcutaneous gas monitoring, and overall attention to homoeostasis of these infants has contributed to the low rate of less severe cicatricial disease in this unit.

Seven VLBW children were noted to have peripheral retinal changes and since there were no analogous retinal changes found in any of the NBW children it is highly probable that these changes represent regressed ROP. The commonest finding was peripheral pigmentation which may represent the region of a previous mesenchymal shunt found in acute ROP or an area of resolved retinal detachment. A retinal tear was noted in an area of lattice in one of these patients and Tasman[36] has emphasised the need to examine these children for peripheral retinal abnormalities. Two cases of myopia and two of squint were also noted among this group with peripheral retinal findings. Previous studies[25 37] have also noted a higher incidence of ocular abnormalities among children with regressed ROP and the need to follow this at-risk group has been stressed.

Despite the improved prospects for VLBW children they still have a significant excess of visual problems. Strabismus, refractive errors, and cicatricial ROP are well recognised causes of visual morbidity among this group but this study has also highlighted the problem of optic atrophy in these children. The prevalence of regressed ROP remains low for the period studied in this neonatal intensive care unit. Severe blinding cicatricial ROP was also rare and this may be accounted for by a smaller number of ELBW children in the study and good perinatal medical care. Children born preterm have an increased risk of long term ophthalmological problems. It is important that they should have regular assessments so that potentially treatable problems such as strabismic amblyopia and refractive errors can be identified.

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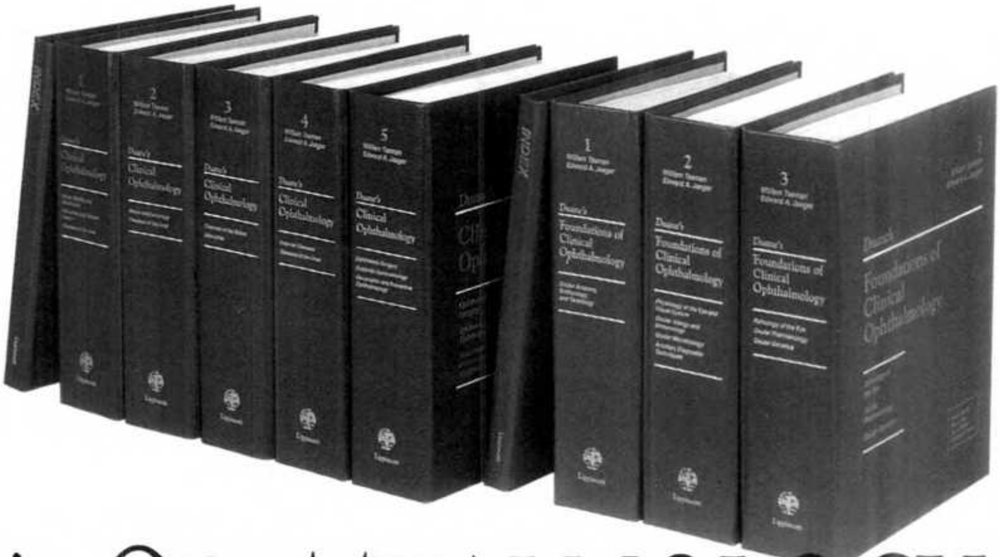

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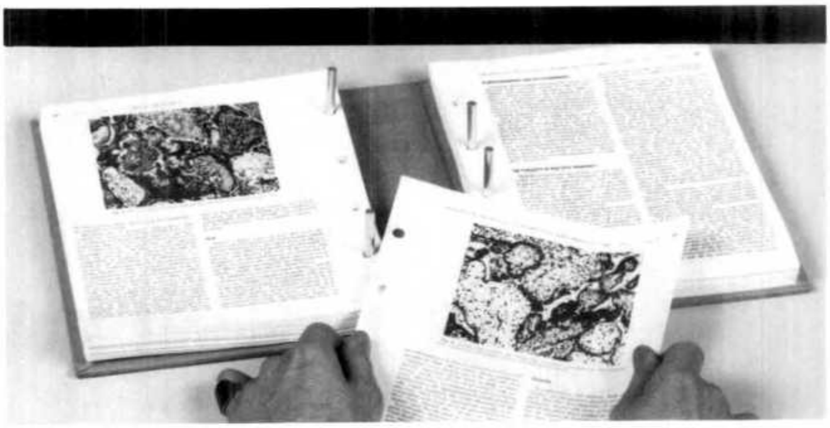

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Vitreon, a short-term vitreoretinal tamponade

Kevin J Blinder, Gholam A Peyman, Uday R Desai, Norman C Nelson Jr, Walid Alturki, Christopher L Paris

LSU Eye Center, Louisiana State University Medical Center School of Medicine, New Orleans, USA
K J Blinder
G A Peyman
U R Desai
N C Nelson Jr
W Alturki
C L Paris

Current address:
Dr Kevin J Blinder, Mason Eye Institute, University of Missouri-Columbia, 1 Hospital Drive, Columbia, MO 65212, USA.

Correspondence to:
Dr Gholam A Peyman, LSU Eye Center, 2020 Gravier Street, Suite B, New Orleans, LA 70112, USA.

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Abstract

This investigation of the liquid perfluorocarbon, perfluorophenanthrene (Vitreon), establishes its safety and efficacy as a short-term vitreoretinal tamponade. We utilised Vitreon as an intraoperative tool and postoperative vitreoretinal tamponade in 16 patients. Proliferative vitreoretinopathy (PVR) (six), giant retinal tear (four), rhegmatogenous retinal detachment (three), retinal detachment with keratoprosthesis (two), and submacular and vitreous haemorrhage (one) were successfully repaired. Vitreon was left in the eye and removed 5 days to 4 weeks postoperatively. Complications encountered included proliferative PVR (five), limited peripheral retinal detachment (three), macular pucker (two) cataract (three), hypotony (two), excessive fibrin reaction (one), and elevated intraocular pressure (one). At the latest evaluation, all retinas are attached with a follow-up of 1·25 to 12 months (mean 6·8 months).

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The denser-than-water vitreous substitutes, first introduced in 1962 by Cibis,[1] are presently experiencing renewed interest. Fluorosilicone oil, with a specific gravity of 1·35, has been shown to be an effective intraoperative tool with a continued tamponading effect postoperatively.[2-4] However impurities present in this substance have made it a less than desirable high specific gravity vitreoretinal tamponade.[5] The liquid fluorocarbons introduced into medicine in 1966[6] were first injected into the vitreous cavity by Haidt *et al* in 1982.[7] Other investigators soon followed, with toxic effects on the retina noted within a short period of exposure.[8-10]

Vitreon, the liquid perfluorocarbon, perfluorophenanthrene, was first evaluated experimentally in 1989.[11] We recently reported the utilisation of Vitreon for intraoperative hydrokinetic retinal manipulation in 15 patients.[12] In this paper we describe our experience with Vitreon as a denser-than-water short term vitreoretinal tamponade under a Food and Drug Administration (FDA) phase I and phase II investigation.

Patients and methods

Vitreon was used in 16 patients to treat various vitreoretinal disorders between December 1989 and December 1990. Informed consent was obtained from all patients. Of the 16 patients, six presented with proliferative vitreoretinopathy (PVR) stages C_3-D_2, four with giant retinal tears, three with rhegmatogenous retinal detachment and peripheral tear, two with retinal detachment with keratoprosthesis, and one with concurrent submacular and vitreous haemorrhage secondary to age-related macular degeneration (ARMD) (Table 1). The ages of our patients ranged from 13 to 81 with a mean of 49·3 years (Table 2). Seven patients were female and nine were male. Preoperative visual acuity ranged from 20/100 to light perception. Six patients had a history of at least one previous vitreoretinal procedure. The lens status preoperatively was divided into six aphakic, five pseudophakic, and five phakic patients.

The surgical technique utilised was very similar to that described for our recent intraoperative series.[13] After the partial air-fluid exchange step, the injection of Vitreon was continued to a suitable level of slightly below the crystalline or intraocular lens, or just posterior to the iris plane in aphakic patients. Subsequent steps, such as membranectomy, relaxing retinotomy, endolaser photocoagulation, and retinal cryopexy were then performed as indicated. In two patients a portion of the Vitreon was removed and replaced with silicone oil (12 500 cSt). The sclerotomies were then closed, and a No 20 band was placed around the eye slightly posterior to the muscle insertions if no previous encircling element was present. In the case of a giant retinal tear, the encircling element was placed more posteriorly. The infusion cannula was removed last, in order to make any adjustments to the intraocular pressure (IOP) and/or the Vitreon level prior to closing.

Postoperative follow-up of the patients consisted of daily examinations until stable, then weekly evaluations until the Vitreon was removed. Observation of postoperative complications, possibly secondary to the presence of Vitreon, resulted in an early removal date. The goal was to maintain Vitreon's tamponading effect long enough for the formation of firm chorioretinal adhesion by laser or cryotherapy, but to remove it prior to the development of potential complications as determined by earlier animal studies.[11] Positioning restrictions were minimal, with the sitting or supine position easily maintained by all patients. Mandatory supine positioning was recommended in only one aphakic patient, without an intact anterior capsule, to decrease Vitreon contact time with the cornea. Vitreon was removed earlier in this aphakic patient and in one patient with a post-

Table 1 Preoperative profile

Proliferative vitreoretinopathy		6
C_3	2	
D_1	3	
D_2	1	
Giant retinal tear		4
Rhegmatogenous retinal detachment		3
Retinal detachment with keratoprosthesis		2
Submacular and vitreous haemorrhage		1
Total		16

Table 2 Patient series

Case	*Age/sex*	*Preoperative diagnosis*	*Pre-operative visual acuity*	*Intra-operative compli-cations*	*Postoperative complications*	*Vitreon removal*	*Follow-up (months)*	*Post-operative visual acuity*
1	58 F	PVR D1	LP	0	0	3 weeks	8	CF
2	21 M	Giant retinal tear	CF	0	Localised RD upon removal	3·5 weeks	8·5	20/70
3	15 F	PVR C3	20/100	0	PVR with redetachment, reoperation Hypotony Cataract	3 weeks – 1st operation 5 days – 2nd operation	9	CF
4	73 M	Vitreous haemorrhage with submacular haemorrhage secondary to ARMD	HM	0	PVR	3 weeks	6·5	20/300
5	55 M	Giant retinal tear	CF	0	Cataract scheduled for ECCE	3 weeks	12	CF
6	74 M	PVR D1	LP	0	0	11 days	10	CF
7	42 F	Giant retinal tear	CF	0	Cataract	7 days	11	20/100
8	18 F	Prosthokeratoplasty with RD	20/300	0	0	2·5 weeks	9	20/100
9	13 M	PVR D1	HM	0	PVR with redetach reoperation	2 weeks	1·5	HM
10	57 M	Rhegmatogenous RD	CF	0	1. Elevated IOP 2. Limited periperal RD 3. Macular pucker; peeled postop	8 days	8	20/80
11	52 F	Prosthokeratoplasty with RD and endophthalmitis	HM	0	PVR with redetach reoperated	4 weeks, 1st operated; presently in eye 8 weeks, 2nd operation	7	20/400
12	81 M	PVR C3	CF	0	1. Macular pucker 2. PVR with redetachment, reoperation	3·5 weeks	7·5	HM
13	70 M	PVR D2	HM	0	0	3 weeks	5	CF
14	70 F	Rhegmatogenous RD with vitreous haemorrhage	HM	0	Hypotony localised RD	4 weeks	2·5	20/300
15	13 M	Giant retinal tear	CF	Silicone oil mix		4 weeks	2	20/80
16	76 F	Rhegmatogenous RD	HM	Silicone oil mix	Excessive fibrin reaction necessitating tPA injection	3·5 weeks	1·25	20/100

ARMD, age-related macular degeneration; F, female; M, male; RD, retinal detachment; Reop, reoperation; CF, counting fingers; HM, hand motion; IOP, intraocular pressure; PVR, proliferative vitreoretinopathy; Redetach, redetachment; LP, light perception.

operative IOP rise (patient 10). Removal of Vitreon in all patients was performed using a three-port system to accommodate the infusion cannula, light pipe, and a 20-gauge flute needle. A Vitreon-air exchange was initially performed, followed by multiple fluid washings to ensure complete removal of the Vitreon. At this time, the decision was made whether to leave balanced salt solution (BSS), sulphur hexafluoride (SF_6) (15–20%), or silicone oil (12 500 cSt) in the eye, depending on what type of postoperative tamponading effect was desired.

Postoperative follow-up upon removal of Vitreon consisted of daily examinations until stable, then weekly, bimonthly, and monthly evaluations for the first 6 months. Those eyes with silicone oil were similarly followed with immediate oil removal for any silicone-related complications. Recurrent retinal detachment, PVR, and significant macular pucker were repaired with further surgery.

Results

All 16 cases were successfully repaired at the conclusion of surgery. Postoperative follow-up revealed recurrent retinal detachment secondary to PVR formation in one case (6%), with the Vitreon still in the eye. This detachment was repaired upon removal of Vitreon at 3 weeks. Four other eyes (25%) developed PVR after removal of Vitreon, with three of these (19%) necessitating further surgery. Three patients (19%) were noted to have limited peripheral retinal detachments, one during Vitreon removal, and two after Vitreon removal. All three retinal detachments were repaired with air-fluid exchange and subsequent photocoagulation. Macular pucker occurred in two patients (12·5%), one necessitating further surgery due to macular distortion, and the other due to concurrent PVR. Cataract formation occurred in three patients (19%); one patient was treated by pars plana lensectomy during Vitreon removal, one is scheduled for an extracapsular cataract extraction, and the third is presently being observed.

Only one patient (6%) was noted to have an elevation of IOP postoperatively with the Vitreon still in the eye. This was possibly an overfill phenomenon, with the Vitreon pushing the iris diaphragm forward, causing an angle-closure component. Postoperatively we removed 0·75 ml of Vitreon at the slit-lamp, with the rest being removed at 8 days in this patient. The timing of removal of Vitreon varied from as early as 5 days to as long as 4 weeks postoperatively. Vitreon was replaced with BSS in 10 eyes, silicone oil in five eyes, and SF_6 in one eye. Of the four reoperations for PVR, three required silicone oil, and one eye still has intraocular Vitreon present after 8 weeks. This last case was a recurrent retinal detachment with PVR complicating a Cardona keratoprosthesis. This patient is being closely followed for the first signs of emulsification, where upon prompt removal will be performed.

Only one patient (6%) was observed to have an excessive fibrinous reaction postoperatively, which was treated by tissue plasminogen activator (tPA) injection. This patient was one of two patients in our series who also had approximately 1–2 ml of silicone oil injected over the Vitreon bubble to provide a tamponading effect for the superior retina. Hypotony (IOP <5 mm Hg) was noted to occur in two patients (12·5%) postoperatively. No cases of corneal decompensation or residual intraocular Vitreon were observed.

Upon latest evaluation all retinas are presently attached (retina flat posterior to buckle), with a follow-up of 1·25 months to 12 months (mean 6·8 months). Postoperative visual acuities range from 20/70 to hand motion vision with improvement noted in 12 (75%), no change in two (12·5%), and a decrease in two (12·5%). An ERG was performed postoperatively on one patient with no significant difference between the operated and the normal fellow eye noted.

Discussion

We recently reported the first human investigation of Vitreon in a limited Phase I FDA-approved evaluation.[12] Fifteen patients with a variety of retinal detachments (six PVR, five rhegmatogenous retinal detachments, two giant retinal tears, one retinal dialysis, one tractional retinal detachment) were successfully treated by intraoperative hydrokinetic retinal manipulation utilising Vitreon. The Vitreon was removed intraoperatively in all cases. Two patients developed PVR necessitating further surgery, and one patient developed hypotony. Residual Vitreon was noted in five patients postoperatively, with the future recommendation of multiple fluid washings to ensure complete removal. One patient developed a postoperative IOP rise, most probably secondary to the use of intraocular gas. No patients demonstrated any unusual inflammatory or toxic reaction secondary to the use of Vitreon. All the retinas were reattached upon follow-up, with a mean duration of 6·3 months.

In the present investigation, we used Vitreon as both an intraoperative and postoperative tool in the repair of various types of retinal detachments in 16 patients. The surgical technique was very similar to that described in our initial series, with the exception of leaving the Vitreon in the eye at the conclusion of surgery. Past animal studies have shown lack of toxicity when left in rabbit eyes for up to 6 weeks,[11] and in primate eyes for up to 5·5 months.[13]

In cases of Cardona prosthokeratoplasty, the occurrence of retinal detachment postoperatively is not uncommon.[14–16] Due to inadequate visualisation and limiting mechanical restriction, retinal detachment repair is very difficult.[15 17] Endophthalmitis also occurs at a higher frequency post-prosthokeratoplasty, complicating one of the two patients in our series. Both patients were treated with pars plana vitrectomy by indirect ophthalmoscopy with Vitreon injection, endolaser photocoagulation, retinal cryopexy, and scleral buckling procedure. The patient with concurrent endophthalmitis was also treated with infusion fluid augmented by gentamicin and clindamycin during vitrectomy. After Vitreon removal, this patient developed PVR and underwent a second procedure with limited membranectomy and Vitreon reinjection. The Vitreon has been present intraocularly for 8 weeks with the retina attached. The other patient underwent only one Vitreon procedure with the retina attached at 9 months follow-up.[18]

PVR was the most common preoperative diagnosis in our series. Past investigations have already shown the efficacy of the liquid perfluorocarbons as an intraoperative tool in the treatment of PVR.[12 19] In the present series Vitreon was utilised to assist in reattachment of the retina intraoperatively and to provide a further tamponading effect postoperatively. This latter effect is most important with inferiorly located retinal pathology. Conventional vitreous replacement with intraocular gas or silicone oil has led to an increased incidence of recurrent PVR in the inferior retina.[20 21] PVR in the inferior retina did not occur in our series, possibly as a result of a lack of pooling of RPE cells or membrane-promoting factors in the inferior gravity-dependent region.

Four patients in our series had a diagnosis of giant retinal tear. Past treatment recommendations have been exceedingly complex.[22 23] The efficacy and ease of the liquid perfluorocarbons as an intraoperative tool in the treatment of giant retinal tears have already been established.[12 24] In the series by Chang and associates, using various perfluorocarbons, posterior slippage of the retinal tear occurred in a few patients intraoperatively during air-fluid exchange, and in one patient postoperatively, leading to a permanent retinal fold.[24] In the present series, the advantage of allowing the Vitreon to remain in the eye postoperatively was demonstrated by the lack of posterior slippage of the retinal tears. One patient was noted to have a localised detachment superiorly upon removal of the Vitreon at 3·5 weeks, which was successfully treated by air-fluid exchange and postoperative laser photocoagulation.

Two patients in our series received a combination of Vitreon and silicone oil in order to provide a postoperative tamponading effect to all quadrants of the retina. One day postoperatively, one patient experienced an excessive fibrinous reaction, necessitating tPA injection into the anterior chamber. In both patients two separate meniscus lines were evident throughout the first 3 weeks, indicating the immiscibility of the two substances. After 3 weeks, however, mixing and

Table 3 Complications

Proliferative vitreoretinopathy		5
Recurrent	3	
New onset	2	
Limited peripheral retinal detachment		3
Macular pucker		2
Cataract		3
Hypotony		2
Excessive fibrin reaction		1
Elevated IOP		1

possible breakdown of the two separate substances were noted. Removal was performed by aspiration via a three-port pars plana system. Thus the dual tamponading effect seemed to last as long as the Vitreon and silicone oil remained apart. Further investigation along these lines seems warranted.

Postoperative positioning restrictions were easy to follow in our series. A supine or sitting position led to the desired tamponading effect. When dealing with lighter-than-water vitreous substitutes such as silicone oil or gas, the mandatory prone positioning can be very uncomfortable, thus leading to lower patient compliance levels. The postoperative complications encountered in our series are similar to those encountered with silicone oil vitreous substitution in the past (Table 3).[21 24]

The FDA-approved investigation of Vitreon has proceeded in a logical stepwise fashion. This report establishes the safety and efficacy of Vitreon as a short-term vitreous substitute. Future investigation will further delineate the indications for the use of Vitreon as an intraoperative tool and short-term postoperative vitreous substitute.

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Ocular circulatory changes following scleral buckling procedures

Akitoshi Yoshida, Hiroyuki Hirokawa, Satoshi Ishiko, Hironobu Ogasawara

Department of Ophthalmology, Asahikawa Medical College, Asahikawa, Japan
A Yoshida
H Hirokawa
S Ishiko
H Ogasawara

Correspondence to:
Dr Akitoshi Yoshida,
Department of
Ophthalmology, Asahikawa
Medical College, 4–5
Nishikagura, Asahikawa 078,
Japan.

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Abstract

The effect of segmental scleral buckling (SB) on ocular circulation was evaluated by measurements of the ocular pulse amplitude (PA) and the ophthalmic artery pressure (OAP). In this study the OAP was defined as the intraocular pressure (IOP) at which the PA disappeared during increasing IOP. Twenty four patients with unilateral rhegmatogenous retinal detachment who underwent SB of varying extent were studied; the unoperated fellow eyes served as controls. Both the OAP and the ophthalmic perfusion pressures (OAP minus IOP) decreased significantly ($p<0.01$) as the area of SB treatment increased. Follow-up measurements showed that the OAP was relatively lower up to 3 months postoperatively than after 3 months, and then remained stable. Our results indicated that SB affects the ocular pulse measurements and may decrease ocular blood flow because of decreased ophthalmic perfusion pressure, probably due to increased choroidal vascular resistance, and that the greater the extent of the SB treatment, the greater the possibility of decreased ocular blood flow.

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Scleral buckling (SB) is a well-established surgical technique for treating rhegmatogenous retinal detachment. The rates of retinal reattachment are satisfactory but complications, although rare, do occur.

It is important to determine whether SB affects the ocular circulation. Using the laser Doppler method in humans Yoshida *et al*[1] reported reduced retinal blood flow in eyes subjected to SB, which was thought to be one cause of postoperative complications. Diddie and Ernest,[2] using the microsphere technique, found that encircling decreased choroidal circulation in rabbit eyes. Mano[3] also reported that choroidal blood flow in rabbit eyes, measured by the hydrogen clearance method, decreased as the degree of constriction applied in the encircling operation increased. Dobbie[4] found decreased ocular pulse amplitude (PA) in eyes that had undergone SB with encircling and speculated that the encircling was responsible for postoperative circulatory alterations. However his speculation was based only on pulse amplitude changes, and uncertainty still remains. More importantly, the effects of segmental SB on ocular circulation in human eyes has not been reported.

In the current study we performed ocular pulse measurements[5-7] in patients who had undergone various degrees of segmental SB. We measured the ophthalmic artery pressure (OAP), defined here as the intraocular pressure (IOP) at which the PA disappeared during increasing IOP, and calculated the ophthalmic perfusion pressure as the difference between it and IOP. The significant correlation between ophthalmic perfusion pressure and the extent of the segmental SB procedure is now disclosed for the first time.

Patients and methods

PATIENTS

The study population comprised 24 patients who underwent SB for unilateral rhegmatogenous retinal detachment (Table 1). Informed consent was obtained from all subjects. All operations were performed by the same surgeon (AY), using general anaesthesia, the same materials (No 276 solid silicone tyre and No 40 encircling silicone band, Mira, Waltham, MA, USA), and the same technique (Schepens implant[8]). No vortex veins were sacrificed and no rectus muscles were removed. No complications arose during SB surgery, and the retinas were reattached completely with a single operation. The fellow eyes were not treated. The treated eyes were classified into subgroups A, B, and C which had SB involving one, two, and three or more quadrants, respectively. In four cases, one each in subgroups A and B and two in subgroup C, postoperative mild choroidal detachment developed although there was no surgical occlusion of the vortex vein. The postoperative refractive difference between the treated eye and the healthy fellow eye was within 3 dioptres in all cases.

METHODS

The ocular pulse was recorded at the corneal surface using the ocular cerebral vascular monitor (Digilab, Cambridge, MA, USA) developed by Langham and co-workers[5-7]; we added a highly sensitive amplifier (Sanei, Tokyo, Japan). Figure 1 shows a typical pulse amplitude (PA) wave recorded from an unoperated eye of one patient (IOP = 14 mm Hg).

Pressure was applied gradually to the sclera of the eye using a Langham pressure cup system (Digilab). The PA and the IOP were monitored simultaneously. Because the PA was expected to

Table 1 Characteristics of patients

Age (range, years)	20–69	
mean (SD)	51·2 (17·1)	
Male: female	14:10	
Extent of scleral buckling procedure	1 quadrant:	8 cases (subgroup A)
	2 quadrants:	9 cases (subgroup B)
	≥3 quadrants:	7 cases (subgroup C)

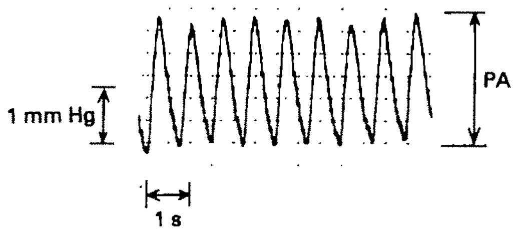

Figure 1 Typical ocular pulse recorded from an unoperated eye of one patient. PA=pulse amplitude.

disappear when the IOP was equal to the systolic OAP, we defined the OAP as the IOP at which the PA disappeared.[6] Figure 2 shows the changes in PA obtained from the same eye as shown in Figure 1 when the IOP was increased. The PA decreased as the IOP increased, and disappeared when the IOP reached 68 mm Hg. In this example, therefore, the OAP was 68 mm Hg.

The PA and the OAP were measured in both eyes of all subjects at 1, 2, 3, 6, 9, and 12 months postoperatively. To minimise variations due to individual differences the untreated fellow eyes served as controls. The PA and OAP ratios were calculated as follows:

PA ratio=PA of treated eye/PA of control fellow eye
OAP ratio=OAP of treated eye/OAP of control fellow eye

The ophthalmic perfusion pressure[6] was calculated as the difference between the OAP and the IOP. We did not calculate the pulsatile ocular blood flow[9] because the pressure-volume relationship[6] may be changed in SB eyes.

The data were analysed using standard statistical methods. Analysis of variance was performed among the groups. Student's t test was performed between group comparisons. Differences were considered significant when the probability value indicated a chance of random occurrence of less than 5%.

Results

Six months after SB the PA of the SB eye was lower in every case than that of the healthy fellow eye. The mean PA (SD) of the SB eyes (0·7 (0·3) mm Hg) was significantly lower ($p<0·001$) than that of the healthy fellow eyes (1·5 (0·6) mm Hg). The mean PA ratio was 0·43 (0·16) at 6 months after the SB. The PA ratio decreased significantly ($p<0·05$) in direct proportion to the extent of the segmental SB procedure (Fig 3) by the analysis of variance.

The OAP of the SB eye 6 months after SB was also lower in every case than that of the fellow eye. The mean OAP of the SB eyes (43·2 (11·8)

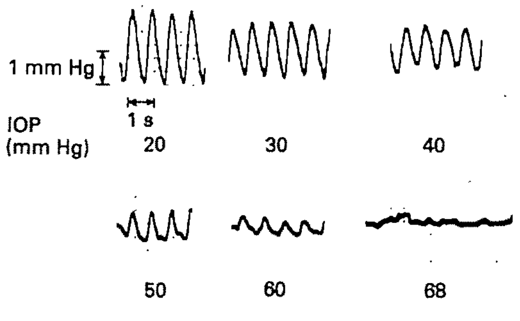

Figure 2 Changes in pulse amplitude obtained from the eye shown in Figure 1 during increased intraocular pressure (IOP). The pulse amplitude disappeared when the IOP reached 68 mm Hg, which was taken as the ophthalmic artery pressure.

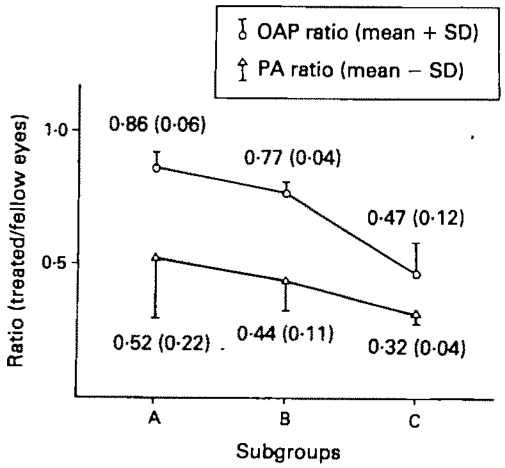

Figure 3 Pulse amplitude (PA) ratio and ophthalmic artery pressure (OAP) ratio 6 months postoperatively for subgroups A, B, and C.

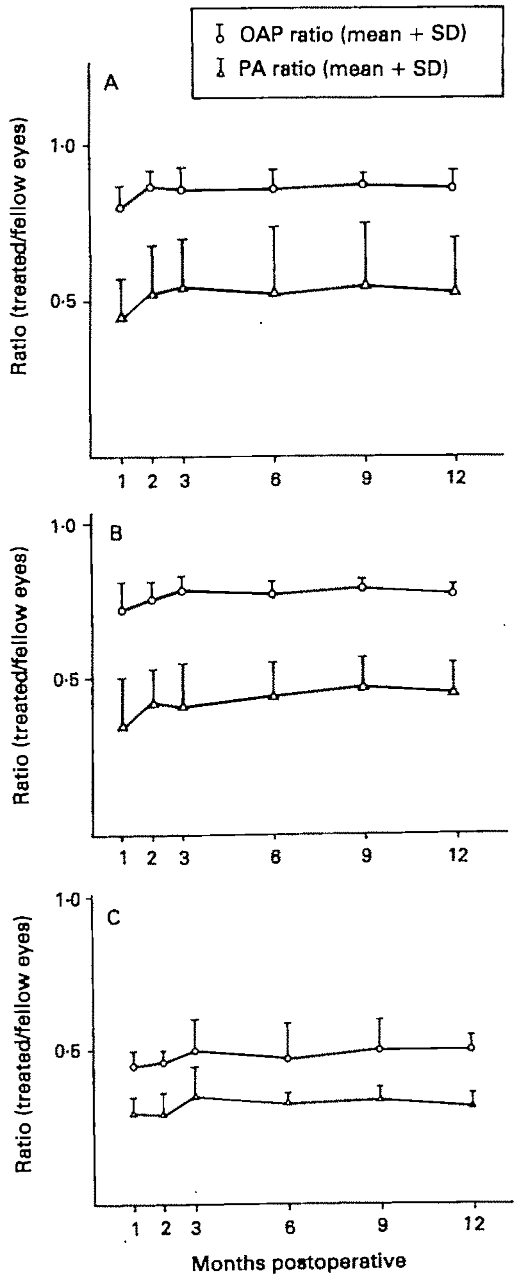

Figure 4 Changes in pulse amplitude (PA) ratio and ophthalmic artery pressure (OAP) ratio subsequent to segmental scleral buckling (SB). A one quandrant of SB in subgroup A (n=8); B two quadrants of SB in subgroup B (n=9); C three or more quadrants of SB in subgroup C (n=7).

mm Hg) was significantly lower (p<0·001) than that of the fellow eyes (61·0 (5·4) mm Hg) 6 months after SB. The mean OAP ratio at 6 months after SB was 0·71 (0·18). Little variation existed in the OAP ratio within each subgroup, and this ratio also decreased significantly (p<0·01) in direct proportion to the extent of SB by the analysis of variance (Fig 3). A statistically significant difference in the ratio also was seen between subgroups A and B (p<0·005) and subgroups B and C (p<0·0001).

The ophthalmic perfusion pressure of SB eyes 6 months after SB (29·0 (11·0) mm Hg) was significantly (p<0·0001) lower than that of the fellow eyes (42·0 (5·6) mm Hg). These values (mm Hg) 6 months after SB were 38·8 (6·6) in subgroup A, 31·3 (3·1) in subgroup B, and 14·7 (5·6) in subgroup C. The ophthalmic perfusion pressure decreased significantly (p<0·01) in direct proportion to the extent of the segmental SB procedure by the analysis of variance.

Changes in the PA and OAP ratios over time are shown in Figure 4. Both ratios tended to be lower 1 to 2 months after SB in all subgroups, then stabilised 3 months postoperatively with virtually no further changes occurring up to 12 months postoperatively. Four cases of mild choroidal detachment occurred postoperatively in subgroups A (one), B (one), and C (two) in which the PA and OAP decreased more than the others in the same subgroup until 3 months postoperatively. Ophthalmoscopically visible choroidal detachment disappeared at 2 months postoperatively in all four cases.

Discussion

We have demonstrated for the first time the relationship between the extent of segmental SB and postoperative ocular circulatory changes. The OAP, as defined in this study, as well as the PA were lower in the SB eyes than in the untreated fellow eyes. The calculated ophthalmic perfusion pressure values were also lower in the SB eyes than in the fellow eyes. Moreover, these three parameters in SB eyes significantly decreased with increasing area of SB treatment, indicating that SB eyes may have lower total ocular blood flow as the SB area increases. This decrease of both OAP and ophthalmic perfusion pressure in SB eyes, compared with the fellow eyes, observed under the same systemic blood pressure in the same person, implies an increase in vascular resistance in the SB eyes due to the surgical procedure.

Our findings of decreased ocular blood flow in human SB eyes are consistent with those of Diddie and Ernest[2] and Mano,[3] who reported decreased choroidal blood flow after SB in rabbits. It has been suggested that venous drainage obstruction (resulting in increased vascular resistance) caused by pressure induced by SB on the vortex vein in the sclera or choroid leads to the decreased blood flow. Using an ocular pulse measurement similar to ours, Dobbie[4] suggested that the decreased ocular circulation resulted from the encircling procedure. However, the amplitude of the ocular pulse itself can be affected not only by ocular circulation changes but also by ocular rigidity changes.[4, 10] Thus, we specifically added another measurement, the OAP as defined here.

Our results indicate that SB affects the ocular pulse measurements and may decrease ocular blood flow because of decreased ophthalmic perfusion pressure (calculated as the difference between the OAP and the IOP). Because OAP can not be measured directly by any available clinical test, we used for this parameter the value of the IOP at which the PA disappeared as defined by Langham and To'mey.[6] Since this is an indirect method for determining OAP, especially based on ocular PA obtained from the operated eye, our values may reflect some artefact and may only appear to be altered.

Nevertheless, our study indicates that ocular blood flow, probably mainly choroidal blood flow (because it constitutes approximately 90% of total ocular flood flow),[11] may decrease after segmental SB and that this decrease seems to be greater if the procedure is more extensive or complicated by choroidal detachment, or both. It is still unclear how this decrease relates to postoperative complications or to changes in postoperative visual function. However, based on our OAP measurements, we at least speculate that as the extent of segmental SB increases, less elevation in IOP would be required to obstruct the choroidal circulation. Thus, for eyes that have undergone relatively extensive SB, adequate care is necessary to ensure that IOP does not increase postoperatively. Although SB is beneficial to achieve retinal reattachment, it is advisable to minimise the extent of the operation as much as possible to avoid complications.

The authors thank Professor Akio Hosaka (Asahikawa Medical College, Asahikawa, Japan), Dr Gilbert T Feke and Mr Stephen N Lipsky (both Eye Research Institute, Boston, MA, USA) for their valuable suggestions.

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British Journal of Ophthalmology 1992; 76: 532–533

Cautery for lower lid entropion

Hosam T El-Kasaby

Abstract

A prospective study was undertaken to evaluate a simple cautery technique for the correction of involutional lower lid entropion in 50 patients. After a 12 month follow-up period all patients were free of entropion. Only one patient needed to have the procedure repeated because of recurrence. The technique was found to be simple, effective, safe, and required very little time and skill.

(*Br J Ophthalmol* 1992; **76**: 532–533)

A multitude of plastic surgery procedures have been reported to correct lower lid entropion[1-6] including Ziegler's galvanocautery puncture initially described in 1909.[1] As many patients with involutional lower lid entropion may be infirm and bed-ridden, it is important that entropion correction can be performed at the bedside. A modification of Ziegler's cautery appeared ideal for this purpose so a prospective study was undertaken.

Materials and methods

Adults suffering from involutional lower lid entropion attending the Wolverhampton Eye Infirmary were consecutively allocated to take part in this study. Informed consent was obtained in all cases. A total of 50 patients were recruited, 22 females, and 28 males aged 58–86 years (mean age 70 years). Only eight patients had previous entropion surgery.

TECHNIQUE

A local anaesthetic (3 ml lignocaine 2%) was injected subcutaneously in the lower lid at the inferior orbital margin near the site of emergence of the infraorbital nerve. This site of injection provided adequate anaesthesia to the lower lid and did not result in excess fluid, which not only could make cauterisation difficult and inaccurate, but also might correct the entropion (Fig 1). Topical anaesthetic drops were instilled in the conjunctival sac. The lower lid was grasped near the centre of its margin with toothed forceps and stretched upwards and away from the eye globe. An electric cautery was used, the wire of which was heated to red hot. A hand held battery cautery is not suitable as it does not reach high enough temperatures. Using the cautery tip, three vertical strokes were made in the centre of each third of the lower lid (Fig 2). These strokes started about 1 to 2 mm from the lid margin and extended to a length of 5 mm. The burns should not reach any deeper than the anterior surface of the tarsal plate. A postoperative dressing was not necessary. Antibiotic ointment was applied to the burns and the patient was asked to use it twice daily for 10 days. The cautery was performed by the same surgeon in all cases (HT E-K).

Results

All patients were reviewed at 2 and 4 weeks postoperatively. After 2 weeks the lower lids of all patients were in good position; none had

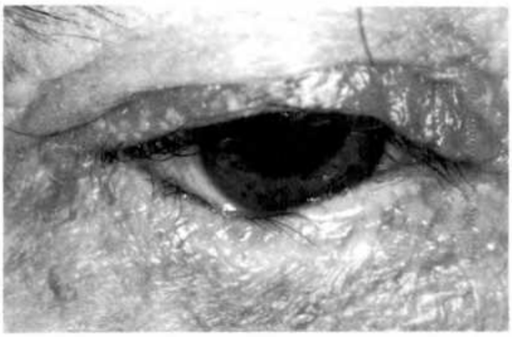

Figure 1 Preoperative lid appearance.

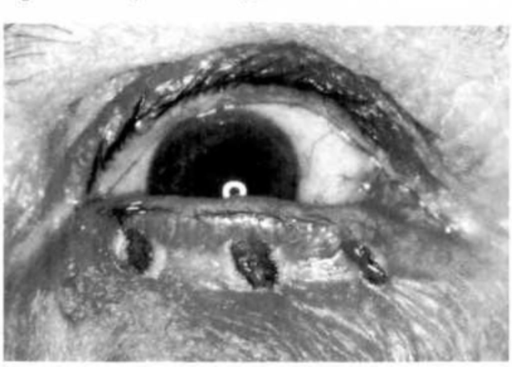

Figure 2 Immediate postoperative appearance.

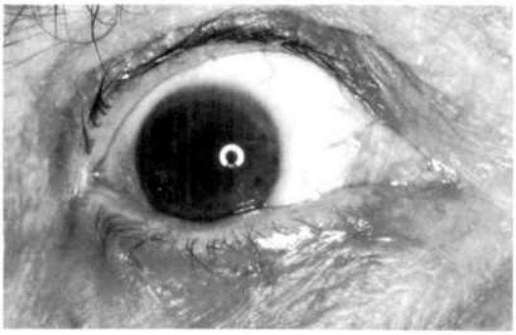

Figure 3 Lid appearance 2 weeks after surgery.

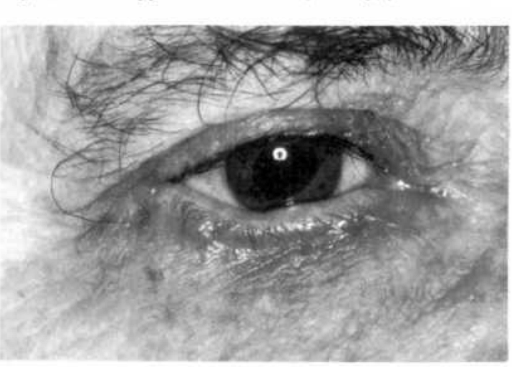

Figure 4 Lid appearance 4 weeks after surgery.

Wolverhampton and Midland Counties Eye Infirmary, Compton Road, Wolverhampton, West Midlands WV3 9QR
H T El-Kasaby

Correspondence to:
Mr H T El-Kasaby, FRCS, FCOphth.

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residual entropion or consecutive ectropion. The lower lid at this stage had three small scabs (Fig 3). After 4 weeks it was difficult to see the cautery scars (Fig 4). No cases of infection were noted. All 50 lower lids were in a satisfactory cosmetic and functional state. The average follow-up period was 12 months (range 6–17 months), with 45 patients followed-up for more than 12 months. All 50 lids remained free of entropion except one which had a recurrence after 7 months. The same procedure was repeated and 10 months later the lower lid remained free of entropion.

Discussion

This procedure works through the formation of three small vertical scars which, on healing, contract and consequently evert the lower lid. These scars also interrupt the continuity of the pretarsal part of the orbicularis muscle thus eliminating its spasm which contributes to the entropion. The scars should also prevent upward migration of the preseptal part of the orbicularis muscle to override the pretarsal part.[7 8]

The procedure described was found to be effective in treating involutional lower lid entropion. After an average follow-up period of 12 months the recurrence rate was minimal. Only one case needed a repeat procedure. A postoperative dressing was not required and postoperative pain was minimal or absent. None of the patients complained about the cosmetic appearance in the immediate or early post-operative period. The procedure requires no sutures and can be easily performed at the bedside.

Lid cautery used as a primary or secondary procedure for the correction of involutional lower lid entropion provides a safe, efficient, and quick method of treatment. The skill required is minimal and the cost is negligible. It is ideal for elderly patients who cannot tolerate lengthy plastic lid procedures, and appears suitable for use in third world countries as the material cost of the procedure is small. However cauterising the skin of black patients might lead to keloid formation and care should be taken.

The author thanks Mr M R Paul, FRCS, FCOphth, for allowing him to publish details of his cases and Mr P Murray, PhD, FRCS, FCOphth, for critically reading the manuscript.

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Prevention of acute postoperative pressure rises in glaucoma patients undergoing cataract extraction with posterior chamber lens implant

James West, John Burke, Ian Cunliffe, Simon Longstaff

Abstract

Acute elevations in intraocular pressure (IOP) commonly follow extracapsular cataract extraction and lens implant in glaucoma patients. Thirty six patients with glaucoma undergoing cataract extraction and posterior chamber lens implantation received one of three treatments. Group 1: 500 mg of Diamox Sustets (acetazolamide) 1 hour preoperatively (10 patients); Group 2: peroperative intracameral Miochol (acetylcholine) (11 patients); Group 3: the above treatments combined (15 patients). IOPs were measured at 3, 6, 9, and 24 hours postoperatively. The average of the maximum pressure rises above the preoperative level over the 24 hour period was greatest for the group receiving acetazolamide only at 8·9 mm Hg; for the acetylcholine group the average maximum rise was 6·3 mm Hg; while the combined treatment group showed a decrease of 0·7 mm Hg. IOP rises of >6 mm Hg were seen in 7% of patients (one of 15) in the combined treatment group, 45% (five of 11) of the acetylcholine group, and 70% (seven of 10) of the acetazolamide group. IOP rises of >10 mm Hg were seen in 7% of the combined treatment group, in 18% of the acetylcholine only group, and in 50% of the acetazolamide only group. A pressure rise >20 mm Hg was seen in one patient receiving acetazolamide only and one patient receiving acetylcholine only. The difference between the acetylcholine group and the combined group for rises >6 mm Hg was significant using the χ^2 test while the acetazolamide group showed a significant difference for rises >6 and 10 mm Hg compared with the combined group. All acute pressure rises were recorded before or at 9 hours following operation except in the combined treatment patient where the rise occurred at 24 hours. To prevent the acute IOP rises seen following cataract surgery with lens implant in glaucoma patients we recommend combined ocular hypotensive therapy.

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Acute rises in intraocular pressure (IOP) commonly follow extracapsular cataract extraction with posterior chamber lens implant surgery (ECCE+IOL),[1-4] figures reported ranging from 10 to 50%. The frequency and degree of such rises appear greater in patients with pre-existing open angle glaucoma, 70% of such eyes having an IOP rise >10 mm Hg or more in a recent study.[5] The already damaged glaucomatous disc may suffer further and worsening and loss of central field following acute IOP rises after ECCE or argon laser trabeculoplasty are well documented.[6-11] The effectiveness of various ocular hypotensive agents, including carbachol, acetylcholine, and several β blockers, at reducing the acute IOP rises have been investigated with some encouraging results,[3 4 12 13] but not in patients with pre-existing glaucoma undergoing ECCE+IOL. Because of the lack of research in this important therapeutic area we undertook a study to compare the effectiveness of two agents, with different mechanisms of action, at reducing the acute postoperative IOP rises. Acetazolamide (Diamox) which reduces aqueous production and acetylcholine (Miochol) which increases aqueous outflow were used alone or in combination.

Material and methods

Ethical committee approval was obtained prior to commencement of the study.

PATIENTS

Any patient with a diagnosis of open angle glaucoma based on an IOP of more than 21 mm Hg prior to treatment and pathological cupping of the optic disc with visual field loss, undergoing ECCE+IOL surgery, were included in the study following informed consent. Patients with previous intraocular surgery were excluded. None of the patients had undergone previous laser trabeculoplasty. All patients were on some form of topical ocular hypotensive agent, some receiving more than one agent.

Patients were randomly allocated to one of three treatment groups. *Group 1:* received Diamox sustet (acetazolamide) 500 mg orally 1 hour preoperatively; *Group 2:* received intracameral Miochol (1:100 solution of acetylcholine chloride in 3% mannitol) at the end of the operation to refill the anterior chamber; *Group 3:* received both treatments. Acetazolamide and acetylcholine were chosen because of their different mechanisms of action, acetazolamide decreasing aqueous production and acetylcholine increasing aqueous outflow; so in theory, when combined one might expect to see a synergistic effect.

A control group of patients with open angle glaucoma undergoing ECCE+IOL and receiving no treatment to prevent postoperative IOP rises was not included as it was felt unethical to do so considering the previously reported frequency of acute IOP rises in the literature with the consequent risk of such rises for an already compromised optic disc.

Department of Ophthalmology, Royal Hallamshire Hospital, Glossop Road, Sheffield S10 2JF
J West
J Burke
I Cunliffe
S Longstaff

Correspondence to:
J West.

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Table 1 *Patient groups*

	Diamox	*Miochol*	*Combined*
No	10	11	15
Mean age	80	78	80
M:F	4:6	3:8	2:13

All IOP measurements were observer blind and made at the slit-lamp by means of a standardised applanation tonometer used for this study only. Preoperative IOPs were taken the day prior to surgery and postoperative readings at 3, 6, 9, and 24 hours following surgery.

Surgery was standardised in all cases to the use of local anaesthesia, corneal section, endocapsular technique, four suture 10/0 nylon closure of the section, and Healonid (sodium hyaluronate) aspiration with a coaxial cannula through the closed section at the end of the procedure.

No complications occurred in any of the surgical procedures and all pupils were adequately dilated. Postoperatively, patients received 2 hourly topical steroid drops and a short acting mydriatic twice daily because of the well known increased risk of uveitis in these patients.

Results

A total of 36 patients were recruited overall and Table 1 shows the number of patients in each group with age and sex distribution.

The disease severity as judged by the type and duration of preoperative topical therapy and the preoperative IOP level was very similar for all groups. Timolol was the most commonly used topical ocular hypotensive followed by pilocarpine. No correlation was noted between preoperative ocular hypotensive therapy or the preoperative IOP level in those patients in whom an acute rise in IOP occurred.

Fibrinous uveitis was noted in a few cases within 3 to 6 hours of surgery, and its presence did not correlate with acute IOP rises.

In our results we looked at the maximum IOP elevation above the preoperative level over the 24 hour study period and the time period of the acute rises. These were compared for the patients in each of the three groups.

The majority of maximum postoperative IOPs were less than the preoperative level for each patient in the combined treatment group, while the opposite was true for the single treatment groups. Individual rises or falls for each patient grouped appropriately and in ascending order are shown in Figure 1.

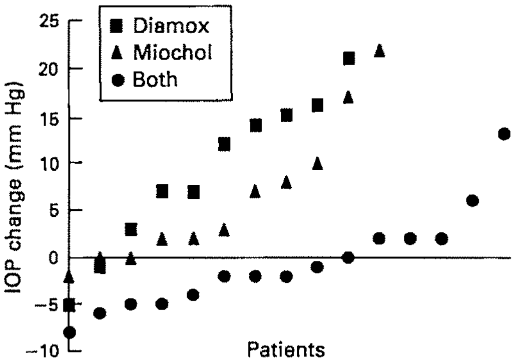

Figure 1 *Individual maximum IOP rises above the preoperative level for each patient.*

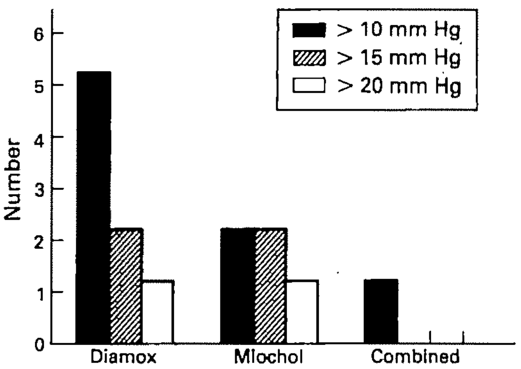

Figure 2 *Number of patients in each group showing IOP rises of >10 mm Hg, >15 mm Hg, and >20 mm Hg over the 24 hour period.*

The mean of the maximum IOP rises was +8·9 mm Hg for the acetazolamide group, +6·3 mm Hg for the acetylcholine group and −0·7 mm Hg for the combined treatment group. The differences between the combined group and the single treatment groups proved significant when compared using Student's *t* test (0·01>p for acetazolamide *vs* combined, 0·02>p for acetylcholine *vs* combined).

Comparing the number and magnitude of IOP rises above the preoperative IOP for each treatment group also showed a marked difference between the combined and the single treatment groups. The difference between the combined group and the acetazolamide group was significant using the χ^2 test for rises >6 mm Hg (0·001>p) and for rises >10 mm Hg (0·01>p), while the acetylcholine group showed significance for rises >6 mm Hg (0·05>p). Figure 2 summarises these findings. For each measurement time the number of IOP rises >10 mm Hg above the preoperative level in each group are shown in Figure 3. Both single treatment groups had at least one patient with a rise >10 mm Hg at 3, 6, and 9 hours postoperatively while none of the patients in the combined group showed a rise of this magnitude at these measurement times. A greater number of rises above 10 mm Hg occurred at the 3 and 6 hour measurement times compared with the 9 hour measurement time for the acetylcholine group, whereas in the acetazolamide group rises were more numerous at the 6

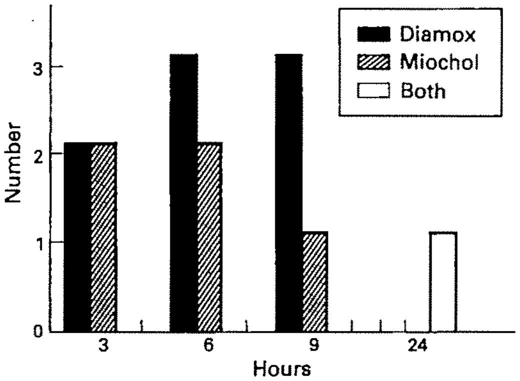

Figure 3 *Number of IOP rises >10 mm Hg recorded at each measurement time for each of the treatment groups.*

and 9 hour measurement times. Only one patient at 24 hours postoperatively had a rise >10 mm Hg and this patient was from the combined group.

Discussion

Early postoperative pressure rises within the first 24 hours in eyes undergoing ECCE+IOL are commonly seen and appear to be maximum at about 6 to 9 hours post surgery.[3,13,14] These early pressure rises are seen more commonly (up to 70% in a recent series[5]) and are of great magnitude in patients with open angle glaucoma undergoing ECCE+ IOL.

A variety of ocular hypotensive agents has been investigated for their ability to prevent these acute postoperative pressure rises with some encouraging results,[3,4,12,13] but no investigation has taken place in glaucoma patients undergoing ECCE+IOL.

Savage *et al*[6] described worsening of the visual field following ECCE in 9·7% of glaucomatous eyes with severe preoperative field loss (split fixation or central field <10 degrees). The potential hazard of acute postoperative IOP rises in an already severely damaged eye with vulnerable optic nerve was suggested and is further supported by other reports in the literature. Hayreh[9] reported 13 cases of anterior ischaemic optic neuropathy following cataract surgery, 11 of which had documented postoperative IOP rises; Kolker[8] reported loss of central vision in 2/23 (8·7%) eyes with advanced glaucoma undergoing cataract surgery (ICCE); Weinreb[10] and Thomas[11] have reported marked loss of visual field and central vision in association with a substantial rise in IOP following argon laser trabeculoplasty.

Despite antihypertensive treatment acute IOP rises were seen in our patients. However, the combination of both agents proved significantly better at preventing the rises than either agent alone. It is interesting that acetylcholine appeared to perform better than acetazolamide as one would expect the meshwork reserve to be low in most of these patients. For both single treatment groups the greatest number of rises above 10 mm Hg occurred at 6 hours post surgery, and the acute rises above 10 mm Hg in acetylcholine treated patients occurred earlier than those in the acetazolamide treated patients. The only rise of more than 10 mm Hg in the combined treatment group occurred at 24 hours post surgery and it is possible that this rise is an escape phenomenon as one would expect the effects of both drugs to have passed by this time.

Some cases of early fibrinous uveitis were seen at 3 to 6 hours following surgery. However patients with open angle glaucoma, who have been on long term antiglaucoma medication and who are undergoing cataract surgery, are known to be at high risk of developing severe postoperative uveitis irrespective of iris trauma during surgery,[15] and none of those patients with an acute postoperative uveitis in this study developed an acute IOP rise.

Another approach to protecting the optic nerve from acute IOP rises is glaucoma triple surgery which has been shown to reduce the frequency and magnitude of early postoperative pressure rises in glaucoma patients.[5,16] However the acute IOP rises are not eliminated by this technique. Furthermore, glaucoma triple surgery is associated with a higher postoperative complication rate and a longer period for visual recovery restricting its use in eyes where topical antiglaucoma medication is effective at maintaining IOP control.[17-21] For the majority of glaucoma patients who have a preoperatively controlled IOP on medical therapy simple ECCE+IOL is the operation of choice as cataract surgery alone is associated with better long term postoperative IOP control.[6,22-24] These patients are at significant risk of developing acute postoperative IOP rises which could cause additional irreversible damage to an already compromised optic disc. An effective means of preventing these acute postoperative IOP rises can only benefit glaucoma patients undergoing ECCE+ IOL.

Our findings suggest a combination of preoperative acetazolamide and intracameral acetylcholine, two agents having different and distinct pharmacological effects on aqueous turnover, is the most effective means of preventing such IOP rises.

We thank our colleagues for their help in recruiting patients for our study.

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Tono-Pen tonometry in normal and in post-keratoplasty eyes

Orna Geyer, Yoram Mayron, Anat Loewenstein, Meira Neudorfer, Leonard Rothkoff, Moshe Lazar

Abstract

Oculab Tono-Pen tonometry was compared with Goldmann applanation tonometry in 82 eyes of 82 patients with normal corneas and in 54 eyes of 54 patients who had undergone penetrating keratoplasty and whose corneas did not preclude the use of the Goldmann tonometer. We found that the intraocular pressure (IOP) in 48% of the eyes with normal corneas and in 57% after keratoplasty has different measurements with Goldmann and Tono-Pen pressures of 3 mm Hg or more. Despite the correlation between the Goldmann tonometer and the Tono-Pen in the group of eyes with normal corneas ($r=0{\cdot}83$) as well as in the group of eyes after keratoplasty ($r=0{\cdot}79$) the Tono-Pen tended to significantly overestimate the Goldmann tonometer reading ($p<0{\cdot}0001$). The mean difference between the two instruments was highest across the lower IOP range (<9 mm Hg) in the group of eyes after keratoplasty. Because the mean absolute values of the paired differences between Goldmann and Tono-Pen measurements varied significantly across all IOP intervals it was not possible to establish a correction factor which could be used when comparing the two measurements. Based on this study the Tono-Pen consistently overestimated the actual IOP in an unpredictable manner. Where possible Goldmann measurements of the IOP are still to be preferred.

(*Br J Ophthalmol* 1992; **76**: 538–540)

The Goldmann applanation tonometer is the 'gold standard' for measuring intraocular pressure (IOP). An alternative tonometer is the Tono-Pen, which is an electronic applanation tonometer based on the same principle as the MacKay-Marg tonometer. It uses a strain gauge that converts IOP into an electrical signal transmitted to a microprocessor where it is analysed for acceptability. The mean value of four acceptable waves is determined, digitalised, and displayed on a liquid crystal panel. The nearest coefficient of variation is also shown on the panel.

This instrument has certain advantages over the Goldmann applanation tonometer. It is portable and compact, can be used regardless of the patient position, is easy to calibrate and operate, has a disposal tip cover which eliminates contamination risks, and the digital readout minimises user bias. Because of its small contact diameter (1·5 mm) the Tono-Pen was recommended for the measurement of the IOP in eyes with irregular corneas.[1]

Our goal was to try to find if the IOP differences between Tono-Pen and Goldmann in normal eyes would be similar in post-keratoplasty eyes which often have large and irregular astigmatism. In addition, we wanted to find a correction factor for the Tono-Pen which could be used to make it clinically comparable with the Goldmann tonometer.

Materials and method

A total of 82 eyes of 82 patients with normal corneas were randomly selected from our clinic population. Fifty four eyes of 54 patients, who had undergone penetrating keratoplasty and whose corneas did not preclude the use of the Goldmann tonometer, were selected from the cornea unit. The Goldmann tonometer and the Tono-Pen were calibrated according to the manufacturer's instructions each day before use.

In both groups of patients one drop of oxybuprocaine HCl 0·4% was instilled in each eye. We performed the examination in uniform sequence, using the Goldmann tonometer first. Measurements were performed on each eye until three consecutive readings were within 1·0 mm Hg. This was followed by Tono-Pen tonometry, where the measurements were repeated until three readings with a 5% range of coefficient of variance shown on the panel were achieved. A disposable latex membrane was applied on the transducer for each patient.

The tests were performed by two ophthalmologists familiar with both tonometers. One used the tonometer and the other the Tono-Pen. This was done in order to eliminate observer bias.

The data were collected and statistically analysed by regression test, paired *t* test and analysis of variance test.

Results

Figure 1 shows the regression line of the Tono-Pen IOPs compared with Goldmann IOPs ($y=0{\cdot}87{,}\times+5{\cdot}63$, correlation coefficient 0·83) in eyes with regular corneas.

Table 1 compares the measurements of both tonometers, in terms of mean paried differences and mean absolute value of paired differences in normal eyes. The analysis is divided into several Goldmann tonometry based IOP intervals. There were significant differences between the two instruments (−3·59 (SD 0·36) mm Hg, $p<0{\cdot}0001$).

Figure 2 displays the distribution of paired IOP differences in the group of normal eyes. Most of the measurements are situated left of the zero, representing overestimation of the Tono-Pen; only 52% of the Tono-Pen measurements

Ichilov Hospital, Tel-Aviv Elias Sourasky Medical Center
O Greyer
Y Mayron
A Loewenstein
M Neudorfer
L Rothkoff
M Lazar

Correspondence to:
Dr Orna Geyer, Department of Ophthalmology, Ichilov Hospital, 6 Weizman Street, Tel-Aviv 64239, Israel

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fell within 3 mm Hg of the Goldmann readings.

There was a significant difference (p<0·0001) in the mean value of the absolute paired difference between the two instruments of each IOP interval (Table 1, Fig 3).

Figure 4 shows the regression line of the Tono-Pen IOPs compared with the Goldman IOPs in eyes with irregular corneas caused by keratoplasty (y=0·71,×+7·08 with a correlation coefficient of 0·79).

Table 2 compares the measurements of both tonometers in terms of mean paired differences and mean absolute value of paired differences in the eyes with irregular corneas. The analysis is divided into several Goldman-based IOP intervals. There were significant differences between the two instruments (−2·96 (SD 5·05) mm Hg, p<0·0001).

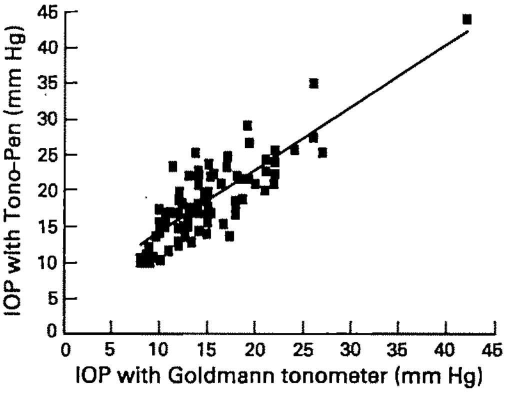

Figure 1 Scattergram and linear regression comparing Goldmann measurements with Tono-Pen measurements on the same normal eyes.

Table 1 Comparison of Goldmann tonometry and Tono-Pen measurements over different Goldmann-based IOP intervals (Goldmann minus Tono-Pen) in normal eyes

	IOP, mean (SD) (mm Hg)	
IOP intervals (mm Hg)	*Paired difference*	*Absolute paired difference*
0–4		
0–9 (*n*=8)*	−2·88 (1·78)	2·87 (1·8)
10–14 (*n*=37)	−4·76 (3·15)	4·77 (3·12)
15–19 (*n*=23)	−3·10 (3·70)	3·74 (3·01)
20–24 (*n*=10)	−1·37 (1·58)	1·77 (1·05)
25–29(*n*=3)	−2·89 (5·50)	4·00 (4·33)
30–34		
35–39		
40–44 (*n*=1)	−2·00	
Overall (*n* – 82)	−3·59 (0·36) p<0·0001	

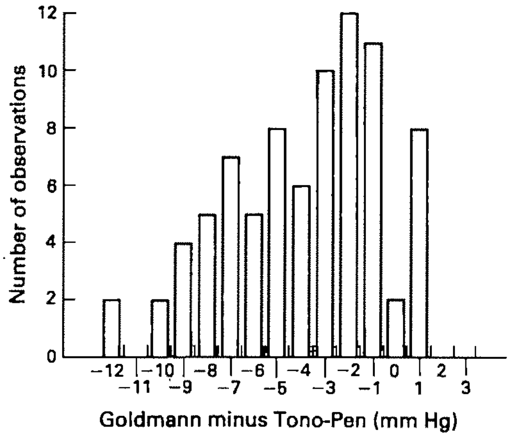

Figure 2 Frequency histogram of paired IOP differences between Goldmann and Tono-Pen measurements in normal eyes. Positive numbers on abscissa indicate that Tono-Pen IOPs are lower than Goldmann IOPs, and negative numbers are higher.

Figure 5 displays the distribution of paired IOP differences in eyes after keratoplasty. Most measurements are situated left of the zero representing overestimation of the Tono-Pen. Only 43·4% of the Tono-Pen measurements fell within 3 mm Hg of the Goldmann readings.

There was a significant difference (p<0·0001) in the mean value of absolute paired difference between the two instruments in each IOP interval (Table 2, Fig 6).

There were no significant differences in the discrepancies between Tono-Pen and Goldmann readings when comparing post-keratoplasty or normal patients (p=0·281).

Discussion

This is the first clinical study to compare the

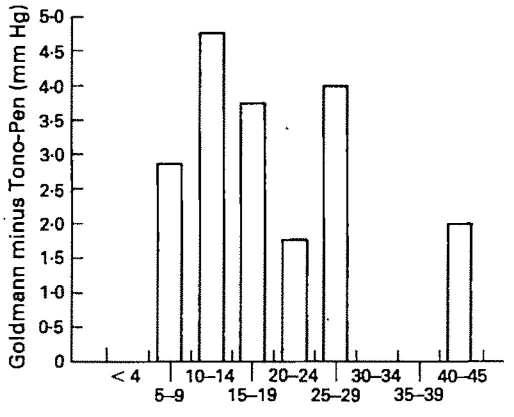

Figure 3 Histogram of the mean absolute value of paired differences between Goldmann and Tono-Pen measurements in different Goldman IOP intervals in normal eyes.

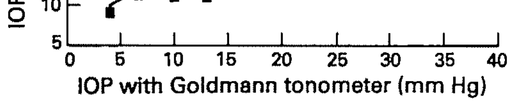

Figure 4 Scattergram and linear regression comparing Goldmann measurements with Tono-Pen measurements on same eyes after keratoplasty.

Table 2 Comparison of Goldmann tonometry and Tono-Pen measurements over different Goldmann-based IOP intervals (Goldmann minus Tono-Pen) in eyes after penetrating keratoplasty

	IOP, mean (SD) (mm Hg)	
IOP intervals (mm Hg)	*Paired difference*	*Absolute paired difference*
0–4 (*n*=2)*	−6·83 (2·54)	6·83 (2·6)
5–9 (*n*=6)	−7·00 (1·35)	1·35 (0·55)
10–14 (*n*=28)	−3·86 (3·26)	2·95 (0·56)
15–19 (*n*=8)	−1·00 (4·69)	3·93 (1·34)
20–24 (*n*=5)	−1·87 (3·17)	1·90 (0·85)
25–29 (*n*=3)	−1·11 (4·67)	1·68 (0·97)
30–34		
35–39 (*n*=1)	7·67	7·67
40–44 (*n*=1)	−5·00	5·00
Overall (*n* – 54)	−2·96 (5·05) p<0·0001	

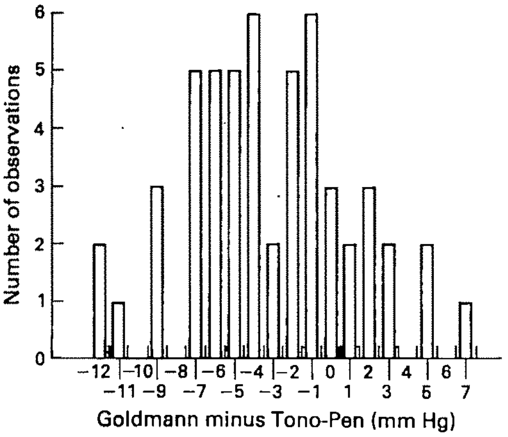

Figure 5 Frequency histogram of paired IOP differences between Goldmann and Tono-Pen measurements in eyes after keratoplasty. Positive numbers of abscissa indicate that Tono-Pen IOPs are lower than Goldmann IOPs, and negative numbers are higher.

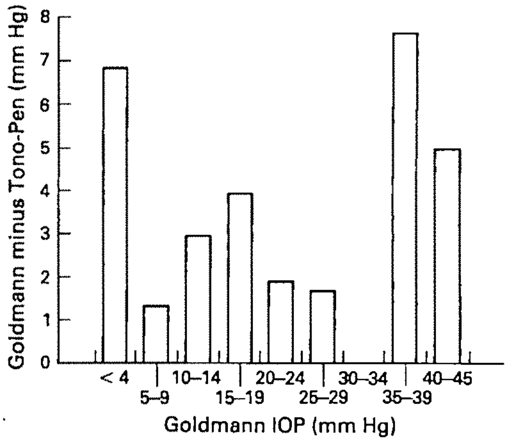

Figure 6 Histogram of the mean absolute value of paired differences between Goldmann and Tono-Pen measurements in different Goldmann IOP intervals in eyes after keratoplasty.

accuracy of the Tono-Pen in eyes with normal corneas with that of eyes which had undergone penetrating keratoplasty. The Tono-Pen was compared with the Goldmann tonometer.

Tono-Pen was found to be as accurate as the Mackay-Marg electronic tonometer in monitoring IOP in eyes with normal corneas and in eyes which had undergone penetrating keratoplasty.[1] However this does not prove the precision of the Tono-Pen, since both tonometers operate the same principle.

In our study there was good correlation between the Goldmann tonometer and Tono-Pen in the group of eyes with normal corneas ($r=0{\cdot}83$) as well as in the group of eyes after keratoplasty ($r=0{\cdot}79$). However, in both eyes, the Tono-Pen tended to significantly overestimate Goldmann tonometry ($p<0{\cdot}0001$).

Kao *et al*,[2] as well as Fenkel *et al*,[3] show that the Tono-Pen tends to overestimate at low IOP intervals (≤9 mm Hg) and underestimate at higher IOPs (≤30 mm Hg), while its measurement corresponds closely to Goldmann applanation tonometry at IOP intervals of 10–19 mm Hg.

Minckler *et al*[4] reported a relatively small overestimation of IOP with the Tono-Pen compared with the Goldmann tonometery and therefore the Tono-Pen could be considered clinically accurate. The large discrepancies in IOP between the two tonometer readings (>6 mm Hg) which they found in their study were attributable to corneal disorders known to interfere with the accuracy of tonometric readings. In our study, the overestimation was much higher than that reported by Minckler *et al*. The mean difference between the two instruments was higher across the lower IOP range (<9 mm Hg) in the group of eyes after keratoplasty compared with eyes with normal corneas. However the mean difference between the Goldmann and Tono-Pen values across the range of IOPs from 10 to 30 mm Hg was not significantly different when comparing both groups.

In order to evaluate how well a single Tono-Pen reading compares with Goldmann tonometry, the mean absolute value of the paired differences was analysed. This revealed that in both groups the Tono-Pen recorded significantly different measurements in all IOP intervals. Therefore, it was not possible to establish a correction factor which could be used to compare the two IOP readings.

Furthermore, IOP in 48% of the eyes with normal corneas and 57% of the eyes after keratoplasty measured differences in Goldmann and Tono-Pen pressures of 3 mm Hg or more and therefore would be considered clinically troublesome.

Based on this study the Tono-Pen is not an accurate means of measuring IOP but usually gives an overestimation with no consistent pattern. If possible IOP should always be measured by Goldmann tonometry.

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Visual evoked potentials after retrobulbar or periocular anaesthesia

Auli Ropo, Pekka Ruusuvaara, Kirsi Setälä

Abstract

The effect of the local anaesthetic agent, etidocaine, on the optic nerve function was examined at regional ophthalmic anaesthesia. Visual evoked potential (VEP) was recorded before and 15 minutes after injection of the anaesthetic agent in 19 patients scheduled for elective cataract surgery (seven retrobulbar and 12 periocular). Both the anaesthetised – that is, the eye to be operated on – and the fellow eye were examined. In the retrobulbar group, two patients displayed non-recordable VEPs while one had virtually non-detectable waves following the anaesthesia. In two retrobulbarly anaesthetised eyes, later peaks were unidentifiable while two other eyes had decreased amplitudes. In the periocular group, in nine patients, there was no clearcut effect on VEP resulting from the anaesthetic. In three patients of this group mild changes in the anaesthetised eyes were found. The differences in the effect of retrobulbarly or periocularly injected anaesthetics on VEP are probably due to the different concentration of the anaesthetic agent around the optic nerve.

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Periocular anaesthesia is an alternative method to the retrobulbar technique for providing local blockade in eye surgery. In the periocular method, the extraconal position of the injection needle is reported to be accompanied by a lower incidence of serious complications.[1,2] On the other hand, life threatening respiratory arrest[3,4] and unilateral or bilateral amaurosis due to toxic or mechanical injury to the optic nerve, although rare, are complications of the retrobulbar technique.[5,6,7] Although some deny that periocular anaesthesia could result in black-out of vision,[1] temporary visual loss caused by the anaesthetic agent has been described both with the retrobulbar and the periocular technique.[8-13] The addition of hyaluronidase to the local anaesthetic solution is believed to increase the transient loss of vision[14] during retrobulbar anaesthesia.

The visual evoked potential (VEP) is an electrical response of the visual cortex to an appropriate visual stimulus. Normal individuals exhibit a variety of complex waveforms arising from flash VEP. In spite of this a unilateral abnormality is detectable with this method.[15] A patient presenting with a cataract alone will demonstrate an intact VEP response. If, however, the cataract is coexistent with – for example, macular degeneration or optic atrophy, then an abnormal VEP becomes evident.[16] Changes in VEP have earlier been detected during general anaesthesia.[17,18] In regional anaesthesia of the eye temporary visual blocks and abnormal VEPs have earlier been reported in a few patients following retrobulbar block.[19,20] On the other hand, in a recent study the VEPs of three patients after peribulbar anaesthesia showed no significant changes.[21]

The purpose of the present study was to compare the effect of a local anaesthetic drug injected either by periocular or retrobulbar route, on VEP.

Department of Ophthalmology, Helsinki University Central Hospital, Haartmaninkatu 4 C, 00290 Helsinki, Finland
A Ropo
P Ruusuvaara
Kirsi Setälä

Correspondence to:
Dr Auli Ropo, Angervotie 4 B 14, SF-00320 Helsinki, Finland.

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Patients and methods

A total of 19 patients scheduled to undergo elective cataract surgery were divided into two groups. Lorazepam (Temesta, Wyeth, USA) 1–2 mg, was given as an oral predmedication 3 hours prior to the administration of regional anaesthesia. One group of seven patients was given a retrobulbar anaesthesia with a semisharp 35 mm needle (2R2, Luer Lock). The other group (12 patients) was given one periocular, inferolateral injection with a sharp 25 gauge, 25 mm needle (Terumo, Belgium). The volume of the injections was 3 ml with the retrobulbar and 4 ml with the periocular technique. The anaesthetic solution in both groups was prepared by mixing 20 ml of etidocaine (Duranest, Suomen Astra, Sweden), 15 mg/ml, with 1 ml of hyaluronidase, 150 IU/ml (Hyason, Organon, Holland). One patient in the retrobulbar group required a further injection of 2 ml because of insufficient akinesia. In the periocular group, four supplemental injections of 3 ml were given superonasally. For orbicular

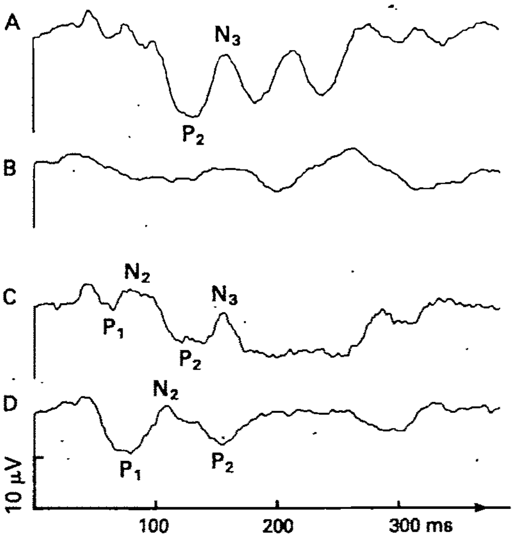

Figure 1 Visual evoked potential (VEP) before (A) and after (B) retrobulbar anaesthesia. VEP is not recordable after the anaesthesia. The fellow, non-anaesthetised eye shows only slightly increased latencies (D) when compared with the pre-anaesthesia situation (C).

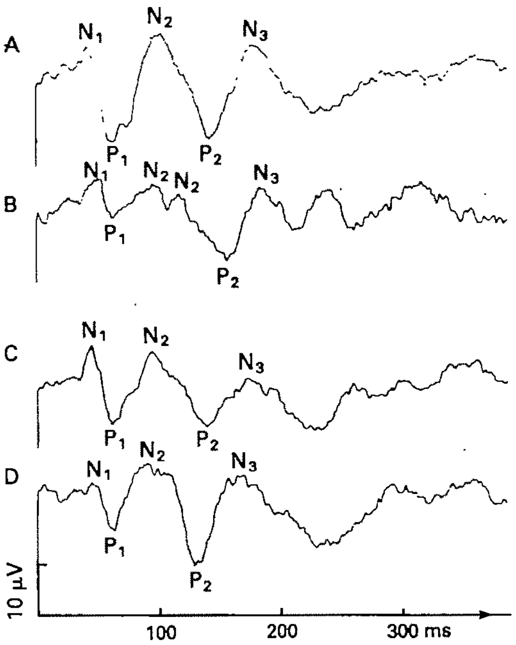

Figure 2 Reduced amplitudes after retrobulbar anaesthesia (B) compared with the pre-anaesthesia responses (A). No clear changes are seen in the fellow eye (C and D).

akinesia, 5 ml of the anaesthetic mixture were injected subcutaneously into eyelids in all patients.

VEPs and subjective light perception with a penlight were examined from both eyes 1 to 3 days prior to surgery. After a dark adaptation of 15 minutes, light flashes of 0·2 J at 1 flash/s were delivered from a Kaiser photostimulator which was placed 20 cm in front of the face. While the one eye was being stimulated, the other was kept covered with a tight patch. The recording electrode was placed in the midline, 2 cm above the inion (O_0 in the international 10–20 system). A common earlobe electrode was used for reference. The response was amplified via an Elema Mingograph. One hundred responses per eye were averaged on-line by a Nokia Channel Analyser LP4840 and recorded with a Houston XY writer. After the regional block was completed, both eyes with dilated pupils were covered. A pressure reducer device (Autopressor, Storz, Germany) was applied to the anaesthetised eye, while the fellow eye was covered with a black patch. When an adaptation time of 15 minutes had passed, subjective light perception was examined and VEP was recorded again from both eyes.

The peaks were identified using N and P for symbols, N being a negative (upward) and P a positive (downward) deflection from the baseline, and put in numerical order according to Harding.[22] The latencies (ms) and peak-to-peak amplitudes of (µV) of waves P_1, N_2, and P_2 were measured from the XY recordings by a neuro-ophthalmologist unaware of which eye had been treated and to which group the patients belonged.

Results

The mean age of the patients in the retrobulbar group was 74·6 (SD 4·7) and in the periocular group 74·1 (SD 2·4) years.

RETROBULBAR GROUP

In the retrobulbar group subjective light perception was normal in five patients 15 minutes after the injection of the anaesthetic agent. One patient in this group had only temporal projection left while a second had lost nasal projection.

Following retrobulbar anaesthesia, the VEPs of two patients were non-recordable although the pre-anaesthesia VEPs were present (Fig 1). One of these patients had a normal subjective light perception while the other had lost her nasal projection. The pre-anaesthesia visual acuities of these respective patients were 0·2 and light perception. The fellow eyes of these patients displayed smaller changes. In the first patient the latencies increased (P_1 by 14, N_2 by 25, and P_2 by 27 ms) while in the second patient only the amplitudes changed (decrease of 6–7 µV). The patient with only temporal projection remaining had hardly recordable responses in VEP showing only waveform N_1 with disappearance of the later waverforms. No changes were found in the fellow eye of this patient.

The late responses disappeared in two patients: in the first, P_2 was no longer identifiable after anaesthesia, while in the second the same was true with N_2 and P_2. The latency of P_1 of the second patient increased by 13 ms. The fellow eyes of these two patients displayed mildly increased latencies of P_2 (11 and 22 ms). In the final two patients, the amplitudes were reduced (Fig 2) and the shape of the waveform N_2 changed from N to W but no changes were found in the latencies. The responses of the fellow eyes did not change.

The two non-recordable VEPs were recorded anew during a check-up visit and were found to be similar to the pre-anaesthesia recordings.

PERIOCULAR GROUP

All patients belonging to the periocular group had normal light perception after the anaesthesia.

After periocular anaesthesia, no patients demonstrated non-recordable VEPs. When

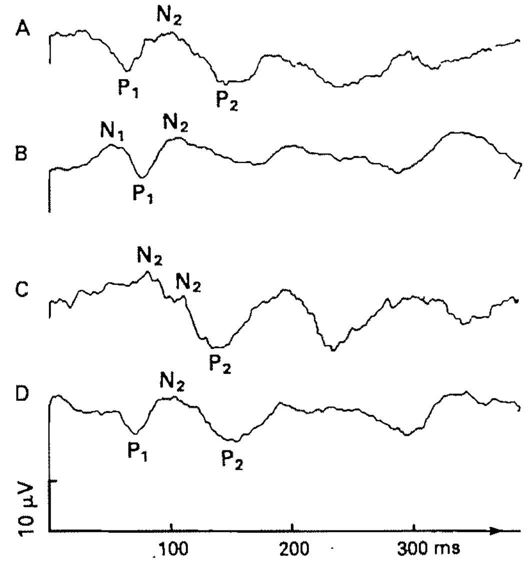

Figure 3 Mild changes after periocular anaesthesia in the anaesthetised eye (A before, B after) as well as in the fellow eye (C before, D after). The late latencies are increased while the amplitudes are decreased.

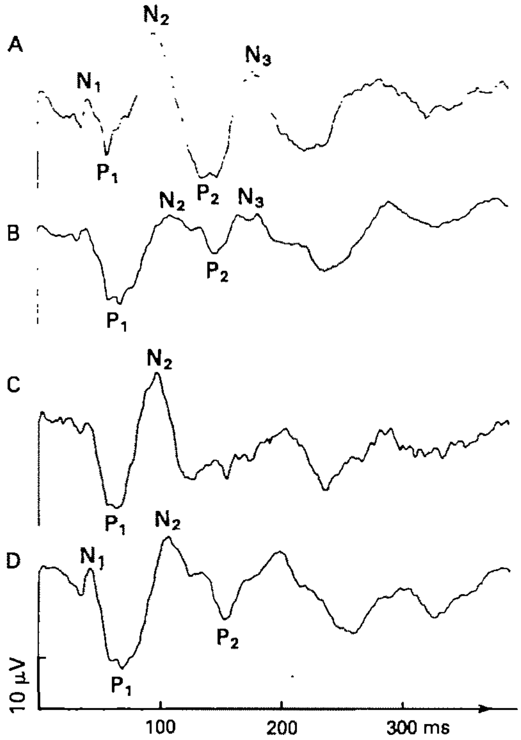

Figure 4 Compared with the pre-anaesthesia situation (A), the latencies are increased and the amplitudes decreased after periocular anaesthesia (B). There are no clear changes in the fellow eye (C and D)

compared with the pre-anaesthesia VEPs, both eyes of eight patients displayed mildly increased latencies and reduced amplitudes (Fig 3). The latencies of P_1, N_2, and P_2 increased by 6 ms (median) in the anaesthetised eyes while the increases in the fellow eyes were 4, 16, and 18 ms (median). The amplitudes of P_1, N_2, and P_2 reduced by 0, 1, and 3 μV (median) in the anaesthetised eyes and by 1, 1, and 2 μV (median) in the fellow eyes.

Of the remaining four patients three manifested with reduced amplitudes (P_1 by 1, 4, and 5 μV; N_2 by 4, 20, and 11 μV; P_2 by 3, 12, and 3 μV) in the anaesthetised eyes with no clear changes in the latencies. No changes were found in the VEPs of the fellow eyes of these three patients (Fig 4). In the last patient of this group, no changes were found in the anaesthetised eye while mild increases in the latencies were detected in the fellow eye.

Discussion

In this study the waves of VEPs were classified according to Harding.[23] Wave P_1 corresponds to a positive peak appearing at around 60 ms (P_{60}), N_2 to a negative peak at about 75 ms (N_{75}), and P_2 to a positive peak at about 100 ms (P_{100}). However, normal values for VEP latencies are dependent on the patient's age and the intensity of the flash. For example, the latency of P_2 increases with age from 114·5 (SD 9·8) (10–19 years) to 134·3 (SD 12·7) (70–79 years) μV/s.[23]

The study reveals that local anaesthetic agents can alter electrical activity of the optic nerve. Periocular anaesthesia (extraconal injection) seemed to have only a slight or no effect on VEP. On the other hand there were obvious changes in VEPs following the retrobulbar (intraconal) injection. A rapid diffusion of the anaesthetic agent from the extraconal to the intraconal space occurs in the periocular technique while the reverse can be seen in the retrobulbar method.[24] When using the retrobulbar technique the concentration of the anaesthetic agent becomes very high around the optic nerve. In the periocular technique however the optic nerve is not reached by all of the injected anaesthetic. The results here support an earlier observation that a transient conduction block is probably caused by the absorption of the anaesthetic agent.[20]

Subjective light perception did not correlate with the total loss of the VEP responses in the retrobulbar group. Unfortunately, the afferent pupillary defect could not be examined because the fellow eye served as control. However in an earlier study, despite the afferent pupillary defect after retrobulbar anaesthesia, 75% of the patients could detect the operating instruments.[12] In the present study all the patients of the periocular group had normal light perception following the injection. This is in contrast to an earlier result[11] in which 22% of patients lost their light perception after their periocular blockade. This might be partially explained by the larger volume of the anaesthetic agent used in that previous study. Subjective light perception in a premedicated patient may also be subject to errors in cooperation.

Premedication and its effect on the electrical activity of the brain might also explain why the VEPs recorded from the fellow eye in 13 of 19 patients showed mild changes. This was especially noticed in the periocular group where eight patients displayed mild, but similar, changes in both the anaesthetised and the fellow eye.

We omitted statistical analysis because of the different size of the study groups. Statistical analysis would also have become unreliable because in three patients most of the waves were unidentifiable after retrobulbar anaesthesia. As it was noticed that retrobulbar anaesthesia with a long needle caused great changes in VEPs the use of that particular method was discontinued.

This study suggests that periocular anaesthesia with a 25 mm needle may be safer for the optic nerve function than retrobulbar anaesthesia with a 35 mm needle. Though the effect of the anaesthetic on the optic nerve conduction is transient it may be deleterious in some compromised (for example, ischaemic, glaucomatous) eyes. Because of this safety factor and the equally good akinesia compared with the akinesia after the retrobulbar technique,[25,26] the periocular method is a good alternative to retrobulbar anaesthesia in eye surgery.

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Immunopathological findings in conjunctival cells using immunofluorescence staining of impression cytology specimens

Christophe Baudouin, Nasser Haouat, Françoise Brignole, Jacques Bayle, Pierre Gastaud

Department of Ophthalmology, Saint-Roch Hospital, Nice, France
C Baudouin
N Haouat
P Gastaud

Laboratory of Hematology, Cimiez Hospital, Nice, France
F Brignole
J Bayle

Correspondence to:
Dr Christophe Baudouin,
Saint-Roch Hospital,
Department of Ophthalmology, BP 319,
06006, Nice Cedex 1, France.

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Abstract

The conventional technique of impression cytology provides a non-invasive method for the evaluation of conjunctival epithelium alterations. Using indirect immunofluorescence procedures two inflammatory markers, class II MHC antigens HLA DR and the receptor to IgE (CD23), were sought in impression cytology specimens obtained from 80 patients. In normal subjects conjunctival epithelial cells did not show any reactivity. Only scattered dendritic cells were found to express class II antigens but not the receptor to IgE. In contrast patients with chronic conjunctivitis of various aetiologies, mainly infectious or allergic, had 40–100% of brightly positive conjunctival cells for one or both antigens. In these cases epithelial cells and goblet cells reacted similarly. Twenty four eyes in 12 patients with idiopathic dry eye syndrome disclosed results similar to those from normal conjunctival specimens. However 18 other specimens from patients suffering from idiopathic tear deficiency but undergoing multiple substitutive treatments for dry eye had moderate to strong positivity for HLA DR and/or the receptor to IgE (20–100% of cells). As these results were independent of the degree of squamous metaplasia the expression of these membrane markers may reflect local inflammation in addition to tear deficiency, possibly due to sensitisation to the eye drops used. These immunocytological techniques thus provide useful methods of investigating conjunctival inflammation and allergy. They may constitute valuable aid in the diagnosis and appropriate treatment of ocular surface disorders.

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Impression cytology is a quick and easy method of obtaining repeated and wide collections of superficial cells from the bulbar conjunctiva.[1-3] It allows quantitative assessment of the number of goblet cells in conjunctiva and a qualitative study of epithelial damage in various conjunctival diseases. This method has been mostly used in dry eye syndrome, a common pathological state in which the non-keratinised stratified conjunctival epithelium progressively loses goblet cells and differentiates into a non-secretory keratinised epithelium.[2]

Although impression cytology provides valuable information concerning dryness-related conjunctival disease, in most cases this method cannot point to the eventual involvement of inflammatory phenomena, isolated or associated with tear deficiency. The eventual functional changes occurring in conjunctival epithelium, especially when cell morphology is not impaired, can only be assessed by immunocytological procedures using monoclonal antibodies to investigate the expression of various cell surface markers. Until now such procedures could not be routinely performed in standard impression cytology specimens, because cell collection is usually made on opaque cellulose membranes which must be chemically cleared before cytological examination. Routine cytological staining can be performed in such a way but not immunocytochemistry, which involves weak antigen-antibody complex formation. Following preliminary reports[4,5] we routinely developed new impression cytology procedures which provided valuable specimens for immunocytochemistry, permitting examination of cell surface changes in conjunctival diseases.

As shown in previous immunocytological studies,[6] class II histocompatibility antigens can be abnormally expressed by a variety of ocular cells in different pathological circumstances. Furthermore, as IgE are known to be directly involved in allergic phenomena and atopic keratoconjunctivitis,[7] an immunocytological study was done using impression cytology specimens of the conjunctiva to investigate the expression of these two inflammatory markers in normal and diseased conjunctiva.

Material and methods

After obtaining informed consent ocular examinations were performed on 80 patients (160 eyes), aged 20 to 85 years. There were 17 randomly selected normal patients, 24 with dry eye syndrome, and 39 with conjunctivitis (of whom seven were affected in only one eye). Diagnosis of dry eye was made on the basis of subjective symptoms, the results of Schirmer test (less than 5 mm for 5 minutes), and tear break up time (less than 10 seconds). Subjective symptoms ranged from mild to severely disabling foreign body sensation or burning. Of these 24 patients only two had the diagnosis of Sjogren's syndrome, on the basis of association with dry mouth and serum autoantibodies (rheumatoid factor or antinuclear antibodies), as described by Pflugfelder *et al.*[8] Another had Stevens-Johnson syndrome, responsible for severe ocular dryness. Although serum antibodies were not obtained in every patient with tear deficiency, the other patients with dry eyes had no clinical or biological sign evocative of systemic disease or local inflammatory impairment.

In the other 39 cases conjunctival specimens were obtained from patients with uni- or bilateral chronic conjunctivitis present for more than 2 weeks before examination. Although in some patients the origin of conjunctivitis was known ocular allergy, bacterial or viral infection, topical medication, or contact lenses, in about 50% no accurate diagnosis could be made.

Impression cytology was performed using two different techniques. The first one was done in 10 normal patients and five with tear deficiency, following the previously described method of Iwata and Burris.[4] For this procedure, Millicel-CM transparent Biopore membranes (PICM03050, Millipore, Bedford, MA) were cut into about 1 cm^2 pieces and pressed on the superior and temporal bulbar conjunctiva, in an area located at 2–8 mm from the limbus. The membranes were applied on the conjunctiva after topical anaesthesia and held there for a few seconds. Cytological and immunocytochemical procedures were then performed directly on the membranes, their transparency allowing microscopic examination. However, as Biopore membranes appeared to be difficult to use routinely because of their thinness and tendency to roll up during manipulations, we investigated a modified impression cytology technique with 0·20 μm cellulose acetate filters (Gelman, Apotechnia), as used in standard impression cytology collection,[1 2] cut into approximately 4×8 mm sheets and placed on the bulbar conjunctiva. In the modified procedure, filters with the detached epithelial cells were firmly applied on gelatin coated slides, and immediately removed, permitting direct cytological and immunocytological examination on the slides. For this technique, it was found that the best cellular collection on glass slides could be obtained when cellulose acetate membranes were slightly wet. As this technique appeared to be more convenient than the Biopore procedure and provided good cytological results, it was adopted for all the other patients of this study. With both procedures, three specimens were collected in each eye (six for each patient), in three closely located but different areas of the superior and temporal bulbar conjunctiva.

Cytological staining was done using 5% cresyl violet in 0·9% NaCl for 30 minutes before rinsing with tap water and microscopic examination. By this method nuclei were stained in violet purple and mucins in pink. Indirect immunofluorescence procedures were performed on the other two specimens, according to previously described methods,[6] using two monoclonal antibodies directed against the monomorphic region of class II histocompatibility antigens HLA DR (OKDR, Ortho Diagnostic System) and a membrane receptor for IgE, CD23 (B6, Coulter Clone).

Primary monoclonal antibodies, in a 1:50 dilution, were incubated for 1 hour, either directly on Biopore membranes or onto glass slides with conjunctival cells. After washing them in phosphate buffered saline (PBS), specimens were incubated for 30 minutes with the secondary antibody, fluorescein isothiocyanate-labelled anti-mouse immunoglobulin antiserum (Dakopatts). They were washed again in PBS, counterstained with propidium iodide and mounted in mounting medium (AF1 mounting medium, Citifluor Ltd) before examination. For microscope examination, Biopore membranes were placed on glass slides and similarly mounted in glycerol containing medium. Nucleus counterstain using propidium iodide was adopted to more easily visualise negative cells and permit a rapid and accurate evaluation of the percentage of positive cells. Percentages of reactive cells were determined by counting at least 200 cells in each specimen. Damaged cells were not considered.

Results

Cytological and immunofluorescence staining using Biopore membranes or cellulose acetate filters showed similar results. Slightly more homogeneous and continuous sheets of conjunctival cells were obtained with Biopore membranes, whereas acetate cellulose filters resulted in large but more often discontinuous and interspaced islets of cells, with more numerous scattered cells. Both types of membranes showed gaps free of cells between spots of variable sizes. Application of membrane to gelatin coated slides did not result in significant cell loss, especially where goblet cell density was concerned, as assessed by the cresyl violet staining procedure. Numerous folds, however, were usually seen when using the very thin Biopore membranes, often accounting for a poor quality of sample collection and cytological examination. Immunocytological patterns were identical with both techniques.

STANDARD CYTOLOGICAL STAINING

As expected, cytological staining of conjunctival specimens from normal patients did not show any morphological anomaly (Fig 1). In contrast, specimens from patients with dry eye syndrome showed various degrees of morphological changes known as squamous metaplasia,[2] including a decrease in goblet cell density (found in all specimens) and more occasionally, a total loss of goblet cells, a flattening of cytoplasm, pyknotic change of nucleus, and even complete keratinisation of conjunctival epithelium. In specimens from patients with chronic conjunctivitis, more heterogeneous results were observed, from normal features to epithelial alterations close to those described above, such

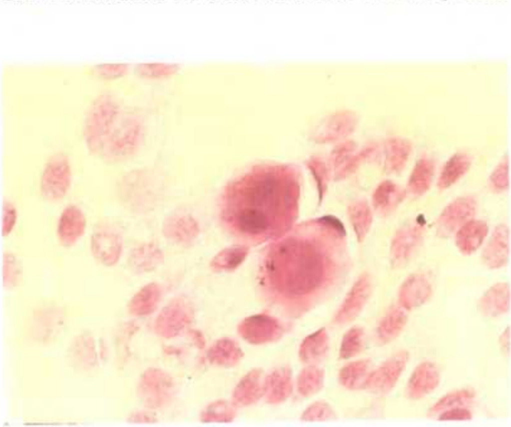

Figure 1 Two goblet cells in an impression cytology specimen from normal conjunctiva (cresyl violet, ×330).

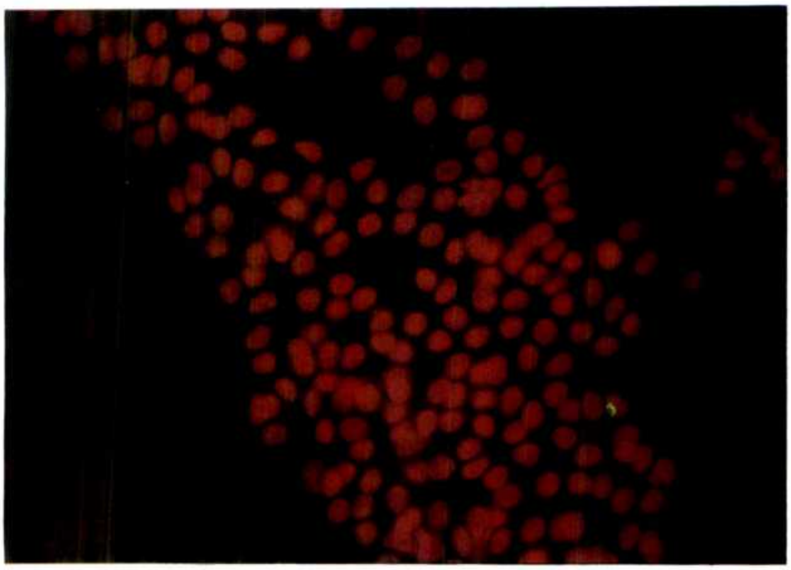

Figure 2 Negative immunofluorescence staining of an impression cytology specimen from a normal conjunctiva with anti-HLA DR monoclonal antibodies. Nuclei are counterstained with propidium iodide and appear in red. Cell membranes do not express HLA DR antigens and cannot be seen by epi-illumination (indirect immunofluorescence procedure, ×340).

as decrease in goblet cell number or changes in nucleus cytoplasm ratios.

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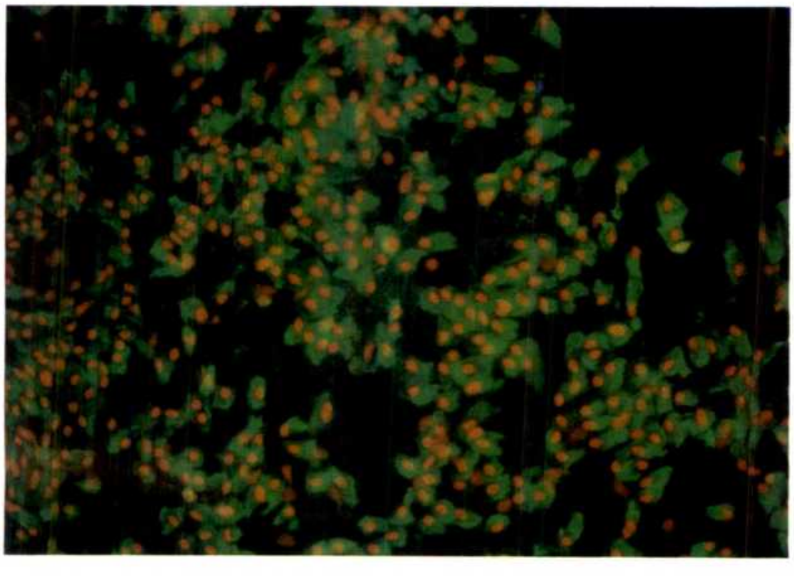
Normal patients

The immunocytological study for class II antigen HLA DR and IgE receptor CD 23 remained negative in all normal conjunctival specimens (Fig 2) except for rare and isolated dendritic cells which strongly reacted with anti-HLA DR monoclonal antibody but not with anti-CD 23 monoclonal antibody. In some specimens, a very weak reactivity was seen on a minority of cells (less than 10%) with either or both antibodies.

Figure 3 HLA DR antigen expression by a large majority of conjunctival cells in a case of chronic conjunctivitis. Positive immunostaining appears in green, whereas nuclei are yellow-orange by superimpression of green immunoreactivity of cell membranes and red staining of counterstained nuclei (indirect immunofluorescence, propidium iodide counterstain, ×240).

Chronic conjunctivitis

The large majority of eyes with conjunctivitis (65 of 71), in contrast, strongly reacted with both monoclonal antibodies (Figs 3 and 4) which were expressed by 40–100% of epithelial cells of the conjunctiva (Table 1). When visible, goblet cells also expressed both markers. In most specimens, both antibodies reacted, but HLA DR positive cells were more numerous and more often found.



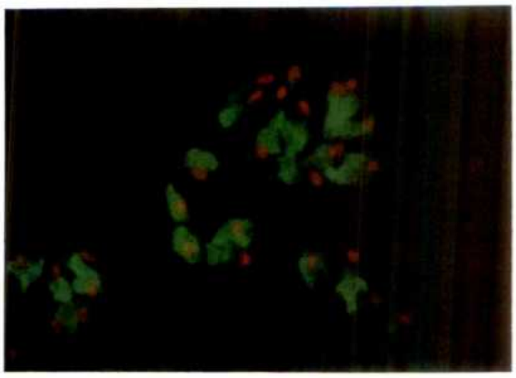


Figure 4 Similar green stained reactivity of conjunctival cells with anti-CD23 monoclonal antibodies in a patient with bilateral chronic conjunctivitis (indirect immunofluorescence, propidium iodide counterstain, ×220).

In cases of unilateral conjunctivitis, a striking difference was seen between both eyes, the normal one remaining negative, while the pathological one strongly reacted. These results were independent of the clinical history, the origin of the conjunctival disease when known, or the degree of cytological anomalies. Conjunctival cells, however, remained negative for both markers in three patients (six eyes) complaining of chronic ocular burning and redness, but without any objective sign of ocular dryness and considered as having chronic conjunctivitis. Two of them had received steroid treatment prior to specimen collection, which could have inhibited the expression of inflammatory markers by conjunctival cells. It was noteworthy that none of the positive specimens were obtained from patients receiving topical steroids.

Patients with tear deficiency

For dry eye syndrome various results were observed. Twenty four specimens with clinical dryness and cytological features of squamous metaplasia remained totally negative for both antigens. Two patients with the diagnosis of Sjogren's syndrome were positive for HLA DR antigens (90–100% of conjunctival cells on both eyes), but not for IgE receptors (less than 10%). The patient with Stevens-Johnson syndrome was strongly positive for both antibodies (95% of positive cells). The 18 other conjunctival specimens from patients with clinical aqueous tear deficiency showed positive but often weaker reactivity (Fig 5) for both monoclonal antibodies (Table 1). Again, the pattern of reactivity and the percentages of positive cells were not related to clinical and cytological features and remained independent of the degree of squamous metaplasia observed in conjunctival cells. Cytological examination did not show a significant difference between negative and positive specimens, as some specimens with total goblet cell loss were negative, whereas very mild forms of squamous metaplasia could be strongly positive. In most cases, percentages of positive cells were similar for both antibodies, but in four eyes the proportion of CD 23 positive cells was higher (60 to 70%) than seen for HLA DR (20–30%). It is noteworthy that, except for two patients (four eyes), all those with positive conjunctival cells had a long term course of topical medication for

Table 1 Immunostaining reactivity of impression cytology specimens from normal conjunctiva and in ocular surface disorders. Mean percentages of reactivity were calculated for positive specimens only

Clinical diagnosis	*No of specimens*	*Mean percentages of cells (SD)*		*Range (%)*		*No of positive specimens*	
		HLA DR	*CD 23*	*HLA DR*	*CD 23*	*HLA DR*	*CD 23*
Normal	(41)*	2 (3·6)	2·5 (4·3)	0–10	0–10	7	5
Conjunctivitis	(71)	79 (22)	54 (29)	40–100	40–100	65	58
Dry eye	(48)	59 (27)	60 (19)	20–100	30–100	24	18

* 17 normal patients and seven contralateral eyes in unilateral conjunctivitis

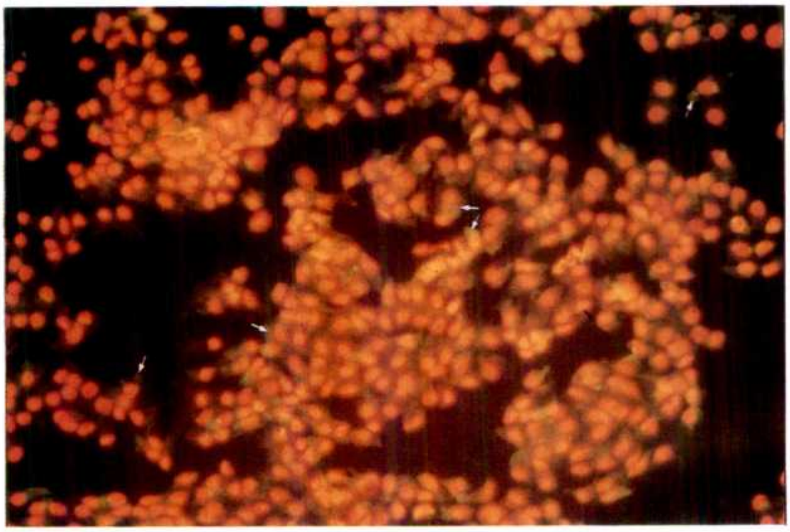


Figure 5 Weaker immunofluorescence reactivity of anti-HLA DR antibodies in some conjunctival cells from a patient with dry eyes. Comparing with Figure 3 or Figure 6, the green immunostaining (arrows) is weak and can be seen only in a minority of epithelial cells. Nuclei are counterstained in orange by propidium iodide (×200).

dry eye syndrome, and that at least two had developed intolerance to their drugs (Fig 6).

Discussion

The technique of impression cytology was first described by Egbert *et al*[1] and provided a simple procedure for cytological assessment of morphological changes occurring in conjunctival cells. These anomalies, designed as squamous metaplasia have been observed in various types of diseases including atopic keratoconjunctivitis, keratoconjunctivitis sicca, xerophthalmia, ocular pemphigoid, Stevens-Johnson disease, or chemical burns.[2] Most of the cytological studies done in conjunctival impression cytology specimens consisted of counting goblet cell density and determining cellular characteristics, such as morphological changes of the nucleus, nucleus cytoplasm ratios, metachromic changes of cytoplasmic colour, and emergence of keratinisation.[2 3] A classification system defining six stages in squamous metaplasia[2] provided an additional application for impression cytology by allowing estimation of the severity of the conjunctival disease and an evaluation of progression or improvement of conjunctival changes.

In addition to morphological analysis, the use of modifications in conventional impression cytology procedure to perform immunocytological studies was thus of interest, by permitting investigation of the expression of various cell markers in normal and diseased conjunctiva. Transferring conjunctival cells from cellulose filters onto gelatin coated slides was a simple way of obtaining a large number of cells on transparent holders, avoiding fixative or chemical clearing agents for filter strips that would not be compatible with immunocytochemistry. We found this procedure to be more convenient than the use of transparent Biopore membranes, first described by Iwata and Burris,[4] and more recently used by Pflugfelder *et al*,[5] because of the excessive thinness of these membranes, which frequently roll up during specimen collection and immunostaining procedures resulting in cell damage and poorer quality of conjunctival cell sheets. Cytological examination of specimens after transfer on glass slides showed a good collection of epithelial and goblet cells, permitting good quality cytological and immunochemical examination. Although both methods provided interesting results, we adopted the transfer procedure for routine collection and standard immunochemistry.

Class II histocompatibility antigens are membrane glycoproteins which are normally restricted to cells of the immune system, such as macrophages, B lymphocytes, and activated T cells, on which they play a major role in the initiation of the immune response to an antigen. Their deviant expression has been observed on various types of epithelial or mesenchymal cells involved in autoimmune diseases,[9 10] and it has been suggested that the aberrant expression of HLA DR by the target cells could play a crucial role in the activation of the immune system and development of inflammatory phenomena.[11] In previous immunocytological studies performed in intraocular proliferative diseases, we found class II antigen expression at the surface of different types of cells, such as vascular endothelial cells in proliferative diabetic retinopathy and retinal or ciliary pigment epithelial cells in proliferative vitreoretinopathy.[6]

Histological examinations of biopsy or autopsy specimens have shown that normal conjunctiva does not express class II antigens.[12 13] Class II antigen expression by conjunctival epithelial cells has been previously found using impression cytology procedures in patients with two autoimmune ocular surface disorders, Stevens-Johnson, and Sjogren's syndrome.[14] In another study, swabs for detection of class II expression by conjunctival cells had been taken from patients with trachoma and demonstrated a strong expression associated with active trachoma,[15] but

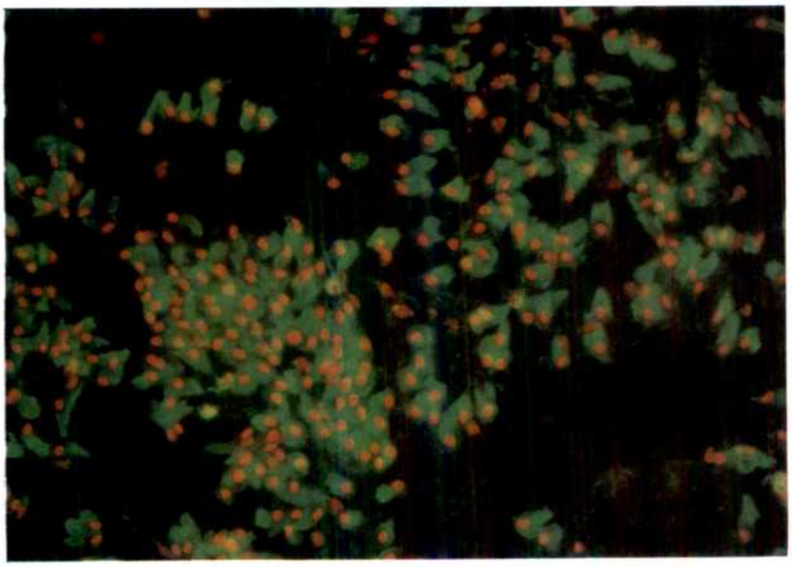


Figure 6 In contrast to Figure 5, conjunctival cells from another patient with dry eyes but showing clinical intolerance to topical medications, strongly express HLA DR antigens on their membrane (green immunofluorescence staining, orange nucleus counterstain, ×200).

until now impression cytology specimens had not been used routinely for the immunocytological investigation of class II antigen expression in ocular surface disorders. The present study confirms the histological assessment that conjunctival epithelium does not normally express HLA DR antigens, except in rare scattered class II positive dendritic cells, which act as antigen-presenting cells to the immune system. We also demonstrated that conjunctival epithelial cells can express class II antigens in various inflammatory conditions clinically defined as chronic conjunctivitis, in which class II antigen expression probably represents more of an epiphenomenon related to local inflammation, than a causal involvement of the conjunctiva.

More interestingly, patients with dry eye syndrome showed different immunocytological features. The immunoreactivity patterns were not related to cytological results and degree of squamous metaplasia. In 24 eyes with dry eye syndrome, conjunctival epithelial cells disclosed immunoreactivity similar to that observed in normal patients. Six eyes of patients with Stevens-Johnson or Sjogren's syndrome and 18 other specimens from patients with idiopathic dry eye syndrome, however, showed an abnormal expression of class II antigens. These patients had clinical diagnosis of dry eye syndrome and various degrees of squamous metaplasia on cytological examination. As HLA DR expression was independent of the stage of conjunctival keratinisation, it may be hypothesised that conjunctival cells were not induced to express class II antigens by ocular dryness itself, but that inflammatory phenomena were associated, possibly resulting from concomitant infection, allergy, toxic effects of topical medications, or direct autoimmune involvement of conjunctiva in Stevens-Johnson or Sjogren's syndrome. Mechanisms of expression of class II antigens are not well known, but interferon-γ has been shown to be a major inducer of such expression by the target cells, both in vivo, in animal models,[16] and in vitro, on a large variety of ocular cells.[17 18]

Similar results were observed with another inflammatory marker, CD 23, which acts as low affinity receptor to IgE, an immunoglobulin which mediates type I hypersensitivity and is increased in serum and tear samples from patients with atopic keratoconjunctivitis.[6] This cell surface antigen has been mainly studied in cells of the immune system on which its expression is enhanced by mediators of inflammation, such as platelet activating factor.[19] Platelet activating factor is a potent mediator that induces platelet aggregation and activation of the immune system, and creates conjunctival inflammation when topically administered to rabbits or humans.[20]

The presence of this receptor at the conjunctival level, as well as HLA DR expression, in some patients with clinical dry eye syndrome, may therefore be related to an associated topical inflammation, that should be treated concomitantly, possibly with anti-inflammatory agents and by avoiding additional sensitisation with inappropriate substitutive medications for tear deficiency. It was not possible in this preliminary report to assess more precisely the consequences of steroid treatments on class II antigen expression by conjunctival epithelium, but it was noteworthy that two of the only three patients with chronic conjunctivitis who exhibited negative reactivity had received topical steroids prior to specimen collection.

Although additional studies need to be performed to evaluate the effect of specific therapeutic agents for topical inflammation, the use of immunocytological procedures in addition to the conventional impression cytology technique may now offer valuable information in conjunctival diseases. Routine immunocytology may be helpful in evaluation and treatment of chronic ocular surface disorders, especially dry eye syndrome, when clinical and current laboratory criteria do not provide the accurate diagnosis and when treatment cannot lead to significant relief.

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Proliferative retinal diseases: myofibroblasts cause chronic vitreoretinal traction

Ronald Walshe, Peter Esser, Peter Wiedemann, Klaus Heimann

Abstract

Myofibroblasts are contractile cells having features intermediate between those of the fibroblast and smooth muscle. Myofibroblasts in preretinal membranes may cause retinal traction in proliferative retinal diseases. In this study, we demonstrate the presence of a chronically contractile subpopulation of myofibroblasts in pre-retinal membranes. Apha smooth muscle actin or desmin, both markers of the myogenically differentiated and chronically contractile subpopulation could be detected in a large majority of the examined membranes. Our findings may serve to explain the cellular basis of vitreoretinal traction in proliferative vitreoretinopathy and proliferative diabetic retinopathy.

(*Br J Ophthalmol* 1992; **76**: 550–552)

Proliferative vitreoretinopathy (PVR) is characterised by the formation of preretinal membranes, the basis of which is cellular proliferation displaying analogues to wound healing and scar formation.[1] Similar cellular membranes can be seen in proliferative diabetic retinopathy (PDR). The membrane contraction can lead to a retinal traction and the resultant detachment is a major complication of vitreoretinal surgery. Myofibroblasts cause retinal traction[2] because of the contractile properties of their cytoplasmic microfilament bundles. Myofibroblasts, having features intermediate between those of the fibroblast and smooth muscle cell, are thought to be responsible for wound contraction and the provision of tensile strength at this stage. However myofibroblasts are not one uniform cell population, for two subpopulations can be distinguished showing clear differences of myogenic differentiation and duration of contraction.[3] The criterion for differential diagnosis is the molecular structure of the intermediate and microfilaments, with the cytoskeletal proteins desmin and α smooth muscle actin serving as markers. Myofibroblasts possessing strong myogenic differentiation contain desmin or α smooth muscle actin and can be shown to cause chronic contraction in pathological tissues, such as hypertrophic scar and fibromatosis.[3] On the other hand, physiological myofibroblasts (for example, in the granulation tissue of normally healing wounds), which, like the pathological ones, always contain the mesenchymal intermediate filament protein vimentin, appear only transiently and cause short term contraction. They never contain desmin or α smooth muscle actin[3,4]

From the foregoing it should be determined if chronically contractile myofibroblasts can be identified in preretinal membranes since these may be of significance in the mechanism of vitreoretinal traction and consequently in the prognosis.

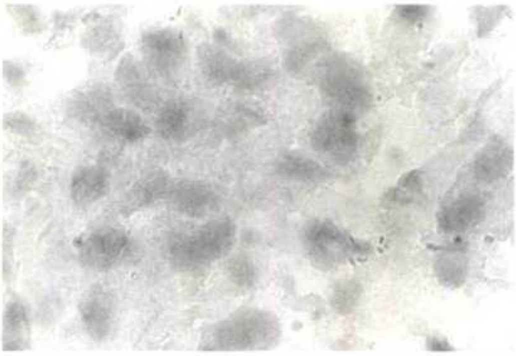

Figure 1 Negative control section treated with mouse IgG instead of specific antibody. Cell nuclei stained dark with haematoxylin and eosin. Magnification ×130.

Materials and methods

Forty five preretinal membranes from patients with eyes having traumatic PVR ($n=25$), idiopathic PVR ($n=10$), and PDR ($n=10$) were obtained during vitreoretinal surgery: these were cooled to −50°C in isopentane and stored at −70°C. Alkaline phosphatase staining was performed on 6–10 μm thick acetone-fixed cryostat sections, using as primary antibodies: monoclonal mouse anti-vimentin (code no M 725, Dakopatts), monoclonal mouse anti-α smooth muscle actin (clone no 1A4, product no A-2547, Sigma) and monoclonal mouse anti-desmin (code no. M 724, Dakopatts).

The primary antibodies were diluted 1:50 with phosphate buffered saline (PBS) and 0·1% bovine serum albumin (BSA) to prevent non-specific attachments. The sections were covered with the diluted antibodies and allowed to react for 60 minutes at 20°C.

Anti-mouse IgG (whole molecule), alkaline phosphatase conjugate ($F(ab)_2$ fragment of affinity isolated antigen specific antibody, product no A-0532, Sigma) was used as secondary antibody in a dilution of 1:50 and incubated for 45 minutes at 20°C. The substrate was made up of: naphthol AS-MX phosphate, free acid (product no N-4875, Sigma), 12 mg,

Table 1 Distribution of the cytoskeletal proteins vimentin α smooth muscle actin, and desmin in 45 preretinal membranes

	Idiopathic PVR (%) (n=10)	*Traumatic PVR (%) (n=25)*	*PDR (%) (n=10)*
Vimentin	100	100	100
α smooth muscle actin	90	80	90
Desmin	30	44	60

Department of Vitreoretinal Surgery, University Eye Clinic Cologne, Joseph Stelzmann Strasse 9, 5000 Cologne, Germany
R Walshe
P Esser
P Wiedemann
K Heimann

Correspondence to:
Peter Wiedemann.

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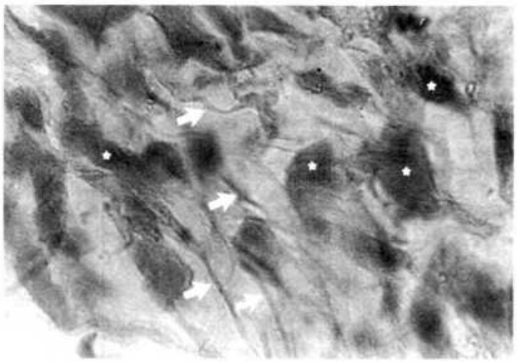

Figure 2 Alkaline phosphatase staining for α smooth muscle actin in a preretinal membrane from a patient suffering from idiopathic PVR. Arrows indicate linearly arranged microfilament bundles which are α smooth muscle actin positive. Counterstaining with haematoxylin and eosin shows cell nuclei (some marked by stars). Magnification ×130.

Fast Red TR (CI 37085, product no F-2768, Sigma), 5 mg, and 4900 μl TRIS buffer, 0·2 M, pH 8·2. Negative controls (Fig 1) were performed on all sections by substituting mouse IgG (product no I-5381, Sigma) for the primary antibodies. Positive tissue controls for the vimentin, α smooth muscle actin and desmin antibodies were obtained by staining the muscular tunic of human small intestine.

Results

The results are summarised in Table 1.

Positive labels for vimentin were observed in all membranes. Alpha smooth muscle actin (Fig 2) was found in 90% of membranes of patients suffering from idiopathic PVR and PDR and in 80% of membranes of those with traumatic PVR.

Desmin (Fig 3) was identified in 30% of slides of idiopathic PVR, 44% of slides of traumatic PVR, and in 60% of cases of PDR.

Discussion

The aim of this study was to draw conclusions about vitreoretinal traction from the occurrence of the cytoskeletal proteins α smooth muscle actin and desmin in preretinal membranes, vimentin serving to identify mesenchymal cells.

Firstly, it must be shown that cells displaying α smooth muscle actin and desmin actually represent myofibroblasts. A safe attribution could be made only on the basis of ultrastructural examinations, but in this context the attribution is compelling for the following reasons; (a) α smooth muscle actin and desmin, which are normally present in smooth or striated muscle only, are characteristic of chronically contractile subpopulations of myofibroblasts.[3] (b) Myofibroblasts have been identified by electron microscopy in PVR membranes.[5] (c) Alpha smooth muscle actin and desmin are atypical of any other cell types present in preretinal membranes: retinal pigment epithelium cells,[6 7] macrophages,[8 9] glial cells,[10] and microglial cells.[11] Therefore, it can be deduced from the distribution of the proteins in PVR and PDR membranes that chronically contractile myofibroblasts with a strong myogenic differentiation appear in the majority of the examined membranes.

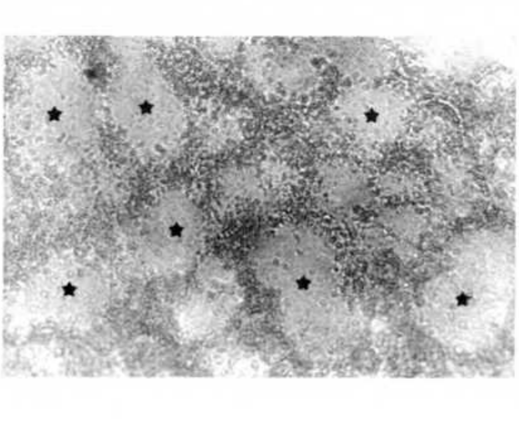

Figure 3 Diffuse intense alkaline phosphatase immunolabelling of desmin in traumatic PVR. Phosphatase converts fast red substrate to a granular red product which appears dark on this photograph. Cell nuclei depicted by stars. Magnification ×130.

Thorough biochemical and ultrastructural examination of these cells resulted in the important finding that myofibroblasts with a strong smooth muscle differentiation are never found in physiological tissues such as physiological granulation tissue and normally healed scar. However they are present in pathological tissues displaying chronic retraction such as hypertrophic scar, fascial and musculoaponeurotic fibromatosis, and intra-abdominal fibromatosis (Gardner's syndrome).[3] For this reason the cytoskeletal proteins used in this study are considered by Skalli *et al*[3] to be markers both with respect to the prognosis of diseases and the effectiveness of treatment.

We believe that our findings can serve to explain the mechanism of vitreoretinal traction in proliferative retinal diseases. Theories of traction so far suggested included cell contraction,[2] cell mediated gel contraction based on the activity of retinal epithelium pigment cells,[12] and hypocellular gel contraction based on the organisation and compaction of collagen fibrils causing anterior loop traction.[13] In our view the presence of myogenically differentiated myofibroblasts in preretinal membranes suggests that cellular (that is, myofibroblastic) contraction does play an important role in the development of vitreoretinal traction.

The distinction between myofibroblastic subpopulations also creates a biochemical basis for examining the different biological behaviour of cells and tissues towards stimulants and inhibitors. It could be shown that various tissues containing myofibroblasts show different patterns of reaction towards substances which cause contraction of smooth muscle cells.[14 15 16] Such a physiological differentiation of myofibroblasts may, just as the above criterion of chronic contraction, be correlated with the molecular composition of cytoskeletal proteins. Finally, the same could hold for the effectiveness of substances inhibiting the proliferation and contraction of preretinal tissues. Examinations of whole membranes of known myofibroblastic differentiation with respect to their contraction behaviour towards various physiological and pharmacological agents (for example, serotonin) might therefore yield pertinent information.

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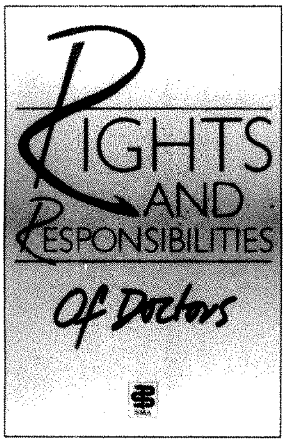

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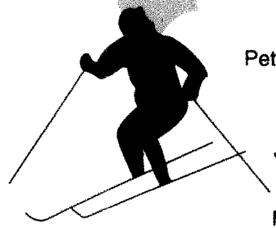

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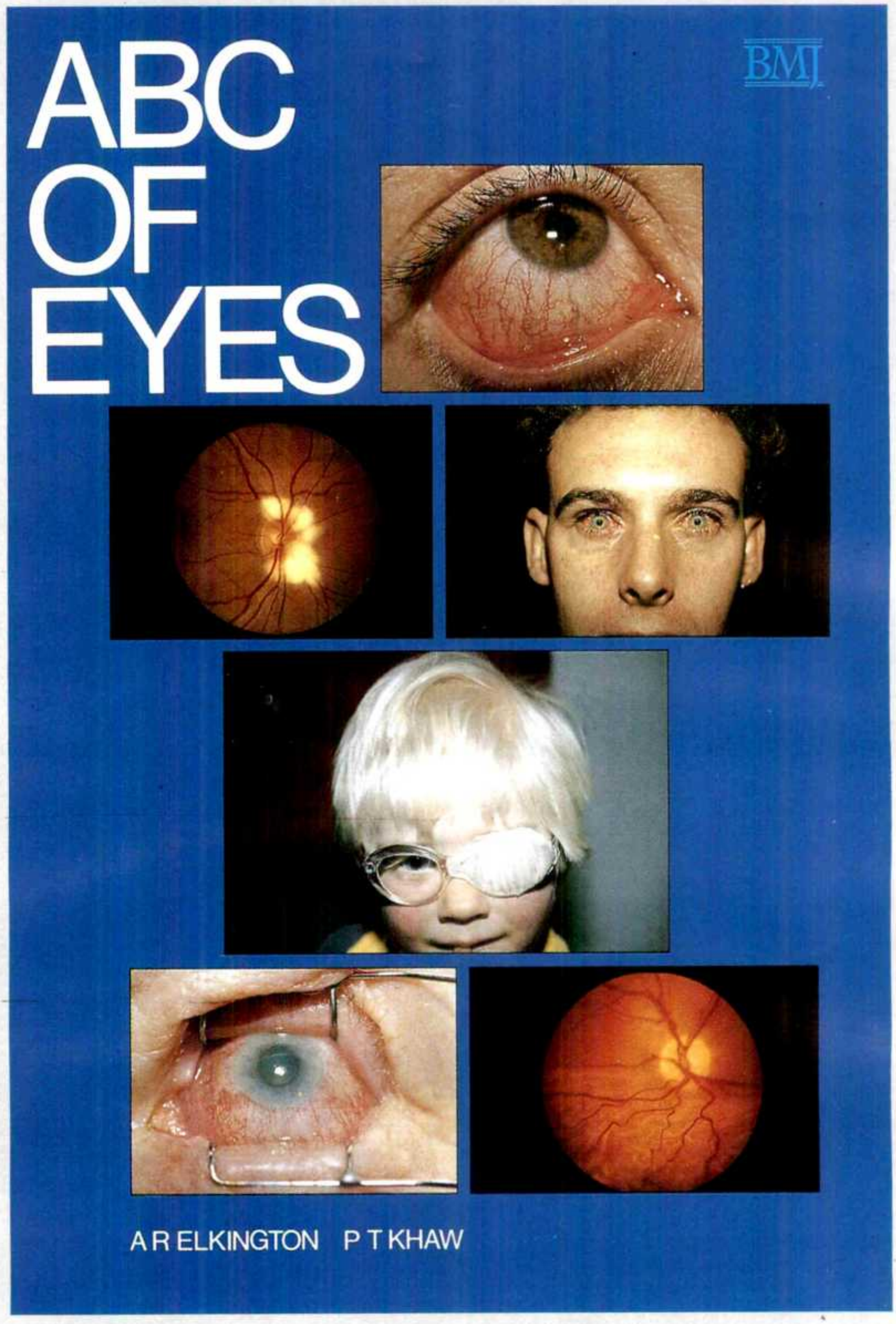

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British Journal of Ophthalmology 1992; **76**: 553–559

Experimental autoimmune uveoretinitis in the RCS rat: the influence of photoreceptor degeneration on disease expression

E G Atkinson, C Edelsten, Eva Kasp, D C Dumonde

Abstract

S-antigen induced experimental autoimmune uveoretinitis (EAU) was produced in the Royal College of Surgeons (RCS) strain of rat which develops a photoreceptor dystrophy within 2 weeks of birth. Animals were sensitised at 60, 90, and 105 days of age: all animals developed disease, but onset was significantly delayed in older (105 day) animals compared with those aged 60 days at sensitisation (p 0·003). Disease was characterised by the early development of complete serous retinal detachment which resolved in a few days: the prevalence of retinal detachment was increased to 80% in dystrophic animals compared with 10% in the congenic, non-dystrophic controls (p<0·001). Anterior uveitis was seen in 17/30 control strain eyes, but in none of 30 dystrophic eyes (p<0·001). Genetically determined photoreceptor and retinal pigment epithelium dysfunction in the RCS rat, which may involve the local accumulation of altered S-antigen, predisposes the dystrophic strain to display an acute retinal detachment in the early stages of EAU. This phenomenon illustrates how biochemical dysfunction of a target organ may influence susceptibility, form, and severity of an experimental autoimmune disease.

(*Br J Ophthalmol* 1992; **76**: 553–559)

Experimental autoimmune uveoretinitis (EAU) is a model of human uveoretinal inflammation, produced by sensitisation of susceptible animal species to discrete retinal proteins, and results in photoreceptor necrosis. Retinal S-antigen, which is confined to the photoreceptors and pineal gland, was the first purified protein demonstrated to produce EAU, and has been the most intensively studied.[1] Autosensitisation to retinal proteins is considered possible because of their anatomical sequestration from the developing immune system. When immunocompetent animals are then sensitised to S-antigen, T helper cells and, later, T cytotoxic/suppressor cells enter the photoreceptor layer and are presumed to produce the characteristic damage.[2] Disruption of the blood-retinal barrier and destruction of photoreceptors promote further access to and recognition of the target antigens by the sensitised immune system. On this basis, continued interaction between the immune system and components of the photoreceptor layer depends on the continuing availability of photoreceptor antigens to the sensitised immune system. Indeed, when the normal turnover of S-antigen at the photoreceptor outer segments is disrupted, as is seen after retinal light damage or dark adaptation, the severity and chronicity of S-antigen induced uveoretinitis is reduced.[3]

Against this background it seemed likely that susceptibility to and severity of EAU could well be affected by coexisting photoreceptor pathology. The Royal College of Surgeons (RCS) strain of rat develops an inherited photoreceptor dystrophy: its abnormal retinal pigment epithelium is unable to phagocytose effectively the photoreceptor outer segment membranes. The photoreceptors start to degenerate within 2 weeks of birth, and cellular debris accumulates spontaneously in the subretinal space: by 2 months of age, the outer nuclear layer is reduced to a thickness of two nuclei. The subretinal debris begins to disappear by 3 months of age, but some may persist for up to 18 months. Eventually, the inner nuclear layer rests on the pigment epithelium, the retinal pigment epithelium (RPE) becomes vascularised and there is gliosis within the remaining retina.[4-8] The black-eyed strain of RCS rat has a pigmented fundus that allows funduscopic and angiographic assessment to be combined.[9] We considered that a comparative study of EAU in congenic, non-dystrophic rats of the same strain would enable evaluation of the influence of photoreceptor dystrophy on disease expression. Accordingly we have studied the form and severity of EAU in RCS dystrophic rats, sensitised at different ages, using congenic, non-dystrophic pigmented rats as controls, and characterising the uveoretinitis by funduscopic, angiographic, and histopathological criteria.

Materials and methods

Fifteen dystrophic and 15 control strain RCS rats were sensitised with 50 µg of retinal S-antigen in adjuvant so as to induce EAU. They were examined initially at 2–3 day intervals from 14–45 days after sensitisation, and then followed-up until day 81: fundus photography and fluorescein angiography were used to document changes in the clinical signs. The animals were then killed on day 86 post sensitisation, and the eyes removed for histological examination. Initial experiments[10] had suggested that the time of onset of disease in dystrophic rats depended on age at sensitisation, and that no such relationship was found in the control strain. Accordingly in this study the dystrophic animals were sensitised at three different ages: 60 days (five rats), 85–90 days (seven rats) and 105 days (three rats). Non-dystrophic controls were sensitised at 60 days (four rats) and 85–90 days (11 rats).

Experiments consisted of documenting the

United Medical and Dental Schools of Guy's and St Thomas' Hospitals, St Thomas' Campus, London
Department of Medical Ophthalmology
E G Atkinson
C Edelsten

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E G Atkinson
C Edelsten
E Kasp
D C Dumonde

Correspondence to:
Dr C Edelsten, Department of Immunology, The Rayne Institute, St Thomas' Hospital, London SE1 7EH.

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ophthalmoscopic and histopathological features of uveoretinitis in dystrophic and control strains at early, intermediate, and late stages of disease; and of examining relationships between the age of onset of EAU and age of rats at sensitisation. Strain differences in the prevalence of EAU or of retinal detachment were evaluated by χ^2 analysis and relationships between time of onset of EAU and age of rats at sensitisation were examined by the Wilcoxon rank sum test.

INDUCTION OF EAU IN RCS RATS

RCS, black-eyed dystrophic rats (rdy/rdy p^+) were obtained from the Central London Polytechnic breeding colony, together with 15 control strain rats (rdy^+/p^+). They weighed 100–150 g and were maintained under non-specific pathogen free conditions. Animals were sensitised in the hind foot pads with 50 µg of S-antigen in Freund's complete adjuvant (FCA: Sigma) in an injection volume of 100 µl. Bovine S-antigen, prepared by previously published methods,[11] was dissolved in phosphate buffered saline (PBS) to 0·5 mg/ml and emulsified with an equal volume of FCA fortified with heat-killed *Mycobacterium tuberculosis* (3 mg/ml: MAFF, Weybridge, England). Simultaneously, 10^6 heat inactivated *Bacillus pertussis* organisms were given intraperitoneally in 0·25 ml PBS.

CLINICAL AND ANGIOGRAPHIC ASSESSMENT OF EAU

Animals were examined by slit-lamp and indirect ophthalmoscopy, after mydriasis with 1% tropicamide (Smith and Nephew). The presence of the following clinical features were recorded separately on each occasion: anterior uveitis, retinal detachment, disc oedema, retinal venous dilatation and periphlebitis, retinal infiltrates, and abnormalities of the retinal pigment epithelium. Selected features were recorded by fundus photography. Fluorescein angiography was performed under Thalamonal anaesthesia, 0·05 ml/kg (Janssen, Oxford). Sodium fluorescein (4%; 0·1 ml) was injected via the tail vein. Angiograms were recorded using a Zeiss fundus camera using Ilford FP4 film.

HISTOLOGICAL PROCESSING

At termination of the experiment, animals were killed by cardiac puncture under ether anaesthesia. Eyes were removed, fixed in 4% glutaraldehyde (EM grade, Emscope Laboratories) for 12 hours, then 10% formaldehyde-saline (BDH); followed by vacuum wax embedding and haematoxylin and eosin staining.

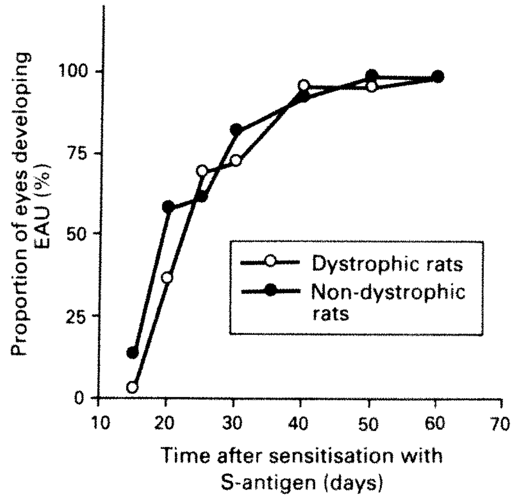

Figure 1 Cumulative onset of EAU in black-eyed RCS dystrophic and non-dystrophic rats. The figure displays the relationship between the proportion of affected eyes, and time after sensitisation with 50 µg bovine S-antigen in FCA together with B pertussis *inoculation (see Methods).*

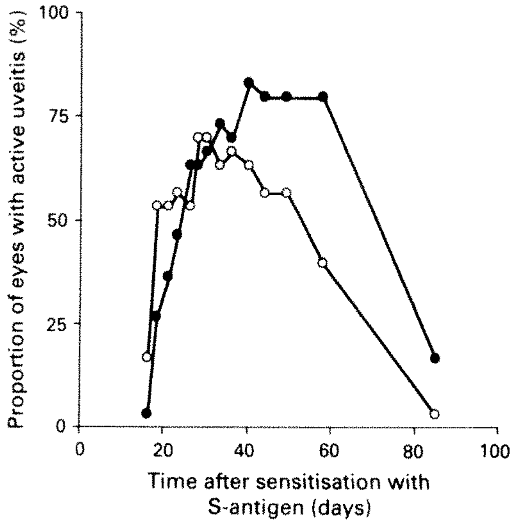

Figure 2 Prevalence of active uveitis in RCS dystrophic rats and non-dystrophic controls compared with time after sensitisation. (○)=dystrophic, (●)=non-dystrophic.

Results

PREVALENCE AND COURSE OF CLINICAL DISEASE IN RCS RATS

All animals developed EAU (Fig 1). The dystrophic strain tended to develop disease earlier, and resolve quicker. At day 18 there were significantly more dystrophic rats with disease than controls (16/30 eyes vs 8/30 eyes: $\chi^2=4\cdot44$, $p<0\cdot05$). but by day 58 there were significantly fewer dystrophic animals with disease compared with controls (12/30 vs 24/30, $p<0\cdot01$) (Fig 2). However, there was no significant difference in the time of disease onset between the dystrophic rats [median 18 days (range 15–46)], and controls [23·5 days (15–55)], or the length of active disease between the two groups: 24 days (3–51) in dystrophic strain vs 29 days (8–61) in control strain. Four non-immunised control strain rats and three non-immunised dystrophic rats did not develop uveitis.

When the animals were grouped according to age at immunisation (Table 1), a tendency for disease onset to be delayed with increasing age at immunisation in the dystrophic rats was found. Animals sensitised at 60 days of age developed

Table 1 Relationship between time of onset of EAU and age of RCS rats at sensitisation with S-antigen

	Onset of EAU (days after sensitisation)	
Age at sensitisation (days)	*Dystrophic strain Mean (SD)*	*Non-dystrophic strain Mean (SD)*
60	18·9 (5·1) (*n*=9)*	26·1 (5·0) (*n*=8)
85–90	22·3 (8·4) (*n*=14)	26·0 (10·5) (*n*=22)
105	30·5 (8·3) (*n*=6)*	ND

**n*=no of eyes

Onset of EAU in dystrophic rats sensitised at 105 days was delayed in comparison with rats sensitised at 60 days (p=0·003, Wilcoxon rank sum test).

Table 2 Clinical features of uveoretinitis in dystrophic and control strain RCS rats

Prevalence of clinical features (%)	*Stage of disease*					
	0–5 days early		*10–15 days intermediate*		*25–30 days late*	
	D	*C*	*D*	*C*	*D*	*C*
Retinal detachment	52	3	0	1	0	6
Anterior uveitis	0	26	0	12	0	0
Retinal vasculitis	37	52	42	41	33	55
Disc oedema	44	38	61	68	35	71
Pigmentary changes	0	3	27	18	93	61

D=dystrophic strain; C=control strains.
Fifteen animals of each strain were examined on three different days during the three time intervals. The prevalence of individual features of disease is expressed as a percentage of 90 observations at these three stages.

uveitis with a mean onset of 19 days, while those sensitised at 105 days of age developed disease at a mean time of 31 days ($p=0\cdot003$, Wilcoxon rank sum test). No such tendency was seen in the control animals. It was concluded that whereas both dystrophic and non-dystrophic rats were susceptible to disease, the dystrophic strain showed a delay in developing disease with increasing age at sensitisation.

CLINICAL FEATURES OF DISEASE IN DYSTROPHIC VS NON-DYSTROPHIC CONTROLS

All animals showed retinal inflammation by indirect ophthalmoscopy; patchy retinal infiltrates, venous dilatation, tortuosity, periphlebitis, and disc oedema. Disc oedema persisted for a few weeks after other signs of retinal vasculitis has subsided. The fundal view was not clouded by vitritis, but severe anterior uveitis prevented funduscopy for a few days in some animals. The fundal appearances were similar in the two strains and resembled the previously reported changes in the Lister rat.[12]

Abnormalities of retinal pigmentation, consisting of patchy hyperpigmentation and atrophy, developed in both dystrophic animals and non-dystrophic controls. There was no difference in the age of onset of pigmentary changes between animals of the two strains, whether sensitised or not (mean age at onset 123 days, 95% confidence interval 117–129).

There was a striking difference in the initial phases of disease between dystrophic and non-dystrophic controls (Table 2): 17/30 eyes of the control strain developed anterior uveitis with miosis and synechiae formation as the initial presentation of the uveitis. In contrast, anterior uveitis was not seen in any of the dystrophic animals ($\chi^2=23\cdot7$, $P<0\cdot001$).

Early disease in the dystrophic strain were characterised by the sudden onset of serous retinal detachment which subsided after a few days; this was followed by other signs of retinal vasculitis similar to those seen in the control strain. Eighty per cent (24/30) of dystrophic eyes developed total serous retinal detachments in the first week of disease compared with 10% (3/30) of control strain eyes ($\chi^2=29\cdot7$, $p<0\cdot001$).

FLUORESCEIN ANGIOGRAPHY AND HISTOLOGY

Fluorescein angiograms showed leakage from the optic disc and areas of intraretinal infiltrates during active disease: no such leakage was seen prior to the development of clinical signs (Fig 3). There was no difference in the angiographic appearances between dystrophic and control strains, and appearances were again similar to those previously reported in the Lister rat.[12] During the phase of serous retinal detachment there was widespread, diffuse leakage of fluorescein in the subretinal space.

Unsensitised dystrophic rats showed the reported changes of photoreceptor dystrophy with extensive loss of the photoreceptor cells and the spontaneous accumulation of debris in the subretinal space (Fig 4A).

All sensitised animals developed an extensive mononuclear infiltrate surrounding the retinal vessels, but minimal choroidal inflammation was seen. The optic disc region showed gross oedema and leucocytic infiltration. In non-dystrophic animals that had developed mild clinical disease, there were local retinal infiltrates but the surrounding retinal architecture remained normal

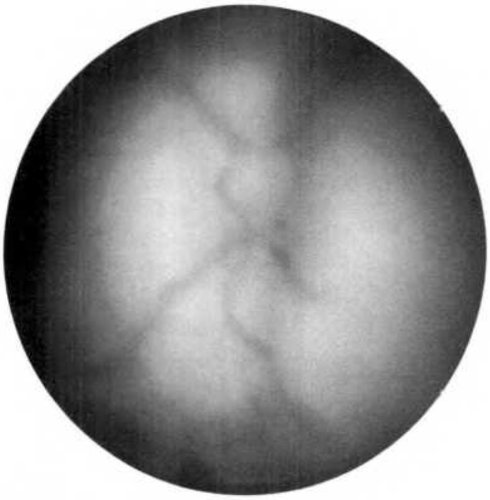

Fig 3A

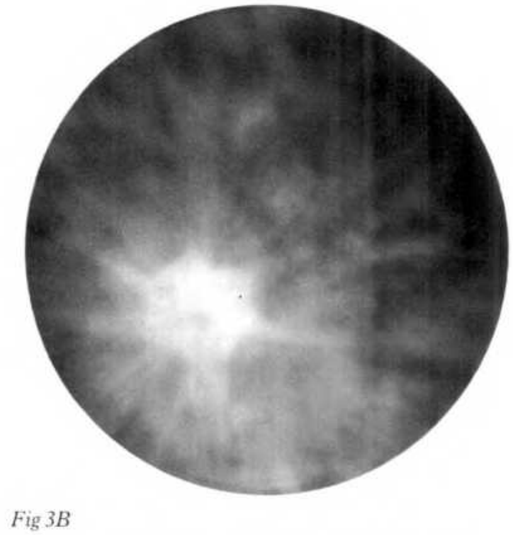

Fig 3B

Figure 3 Fundus photographs and fluoroscein angiograms of dystrophic rats showing clinical features of EAU. (A) serous retinal detachment; (B) normal optic disc; (C) swollen optic disc with dilated retinal vessels; (D) peripheral retinal phlebitis; (E) and (F) are angiograms corresponding to the areas shown in (C) and (D), demonstrating dilated vessels and capillary and venous leakage.

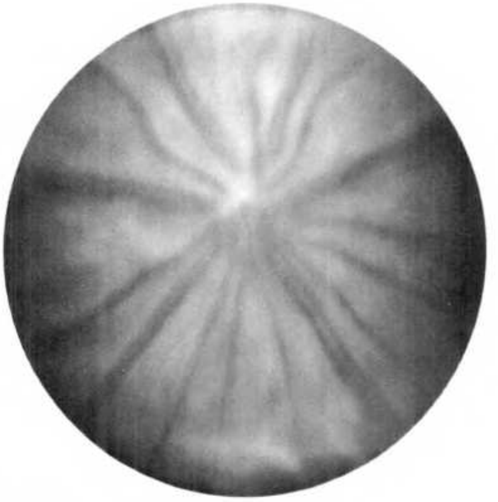

Fig 3C

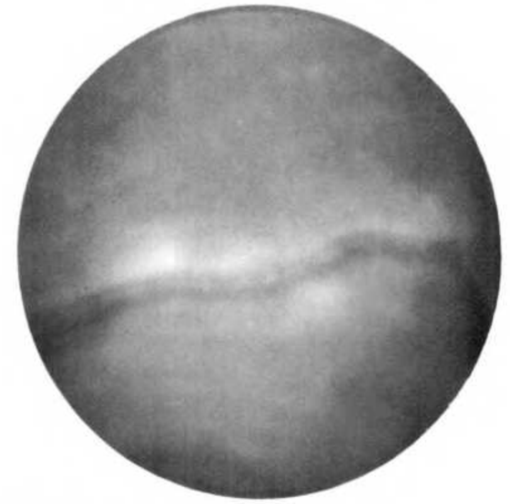

Fig 3D

(Fig 4B). In the dystrophic animals leucocytic infiltration into the retina was characteristically diffuse rather than focal and of greater severity than in the non-dystrophic strain. In the late stages of EAU dystrophic animals showed loss of subretinal debris with thinning of the neural retina; a small inflammatory infiltrate remaining in the inner retina at a time when funduscopic evidence of disease was no longer evident (Fig 4C). In late EAU there were extensive areas of gliosis accompanied by neovascularisation in the dystrophic retina (Fig 4D). Although similar changes could be seen in the control strain, they were much less widespread than in the dystrophic strain. In the anterior segment there was mononuclear cell infiltration of the ciliary body and occasionally evidence of posterior synechiae. In an earlier series of experiments,[10] animals were sacrificed during the early stages of disease. Figure 4E shows the detached retina of a dystrophic rat sensitised according to an identical protocol at the age of 60 days. The animal was sacrificed 14 days after sensitisation and 3 days after the onset of extensive serous detachments. Large clumps of granulocytes and mononuclear cells are seen in the subretinal space.

Discussion

In this study we have demonstrated that the RCS rat is suceptible to EAU after sensitisation with retinal S-antigen, though older animals with photoreceptor dystrophy show a delay in disease onset. The presence of photoreceptor dystrophy was associated with a marked alteration in the form of early clinical disease from an acute anterior uveitis to that of a complete serous retinal detachment. As the two strains of rat differed only by the presence or absence of the photoreceptor dystrophy gene(s) we presume that these differences in disease expression were not due to differences in immune responsiveness or to differences in ocular vascular anatomy between the strains. In general the photoreceptor layer is the site of the most profound T lymphocyte infiltrate and retinal cell destruction in EAU, while in the Lewis rat initial T lymphocyte infiltration occurs in the ciliary body.[13] The route and severity of early cellular infiltration into the eye in EAU varies with the ocular vascular anatomy, the dose of antigen, the composition of adjuvant as well as between strains of the same species.[14-18] In EAU, fluorescein angiography and electroretinography demonstrate that the cellular infiltrate is preceded by a period of increased retinal vascular permeability and abnormal photoreceptor function, and there is

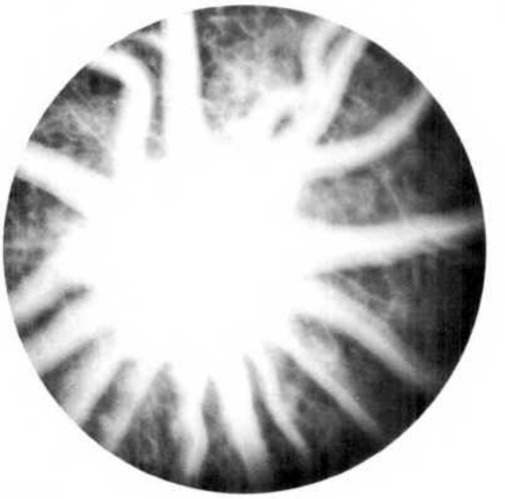

Fig 3E

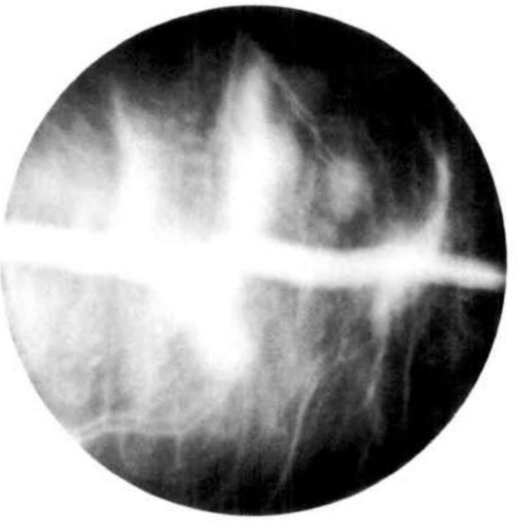

Fig 3F

Figure 4 Histology of posterior segment changes in EAU produced in dystrophic and non-dystrophic RCS rats (haematoxylin and eosin). (A) Section from non-sensitised dystrophic rat, 60 days old, showing extensive loss of photoreceptor cells and their replacement with amorphous debris (×130). (B) Section from non-dystrophic rat 86 days after sensitisation. There is a focal outer retinal infiltrate with adjacent disruption of the RPE. The surrounding retina shows normal photoreceptor layer morphology (×150). (C) Section from dystrophic rat with clinically inactive disease, 86 days after sensitisation. There is total loss of photoreceptor layer and associated debris. The inner retina is disrupted and there is irregularity of pigmentation at the RPE (×150). (D) Area of neovascularisation in dystrophic rat, 86 days after sensitisation (×150). (E) Section taken during the phase of acute retinal detachment, 14 days post-sensitisation in a dystrophic rat. There is a marked serous elevation of the retina with subretinal aggregates of mononuclear cells and granulocytes. A mild infiltrate is present in the outer retina (×66).

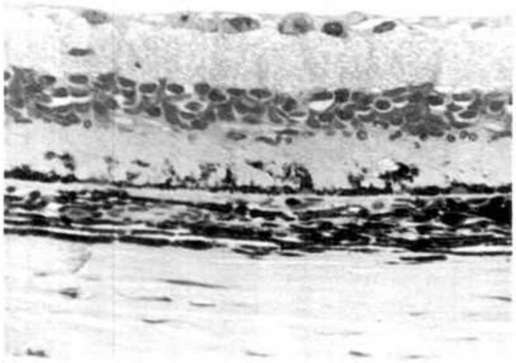

Fig 4A

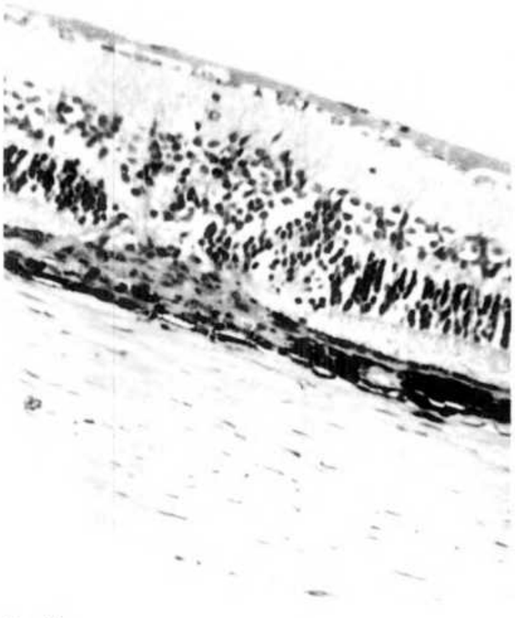

Fig 4B

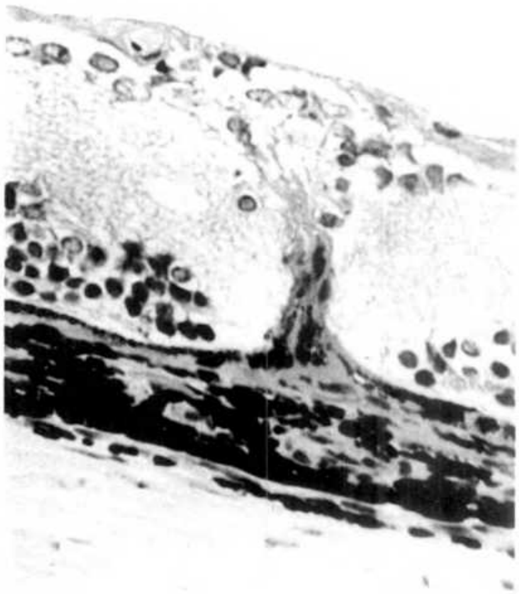

Fig 4D

some evidence that humoral factors may be responsible for those early changes.[12,14-19] Retinal detachment is known to occur during EAU in species other than the rat[16] where it is usually adjacent to areas of choroiditis and to RPE proliferation; and where the subretinal fluid contains lymphocytes and macrophages derived from the choriocapillaris. Extensive retinal detachment with subretinal granulocyte exudation can occur in hyperacute EAU in the Lewis rat, induced by high-dose sensitisation with S-antigen, where it is considered to be immune-complex mediated.[16]

The retina of the RCS rat has three characteristics which may predispose it to develop retinal detachment so readily in EAU. Firstly, normal adhesion of the retina to the pigment epithelium is compromised by the loss of interdigitations between the photoreceptors and RPE, as the outer segments degenerate and

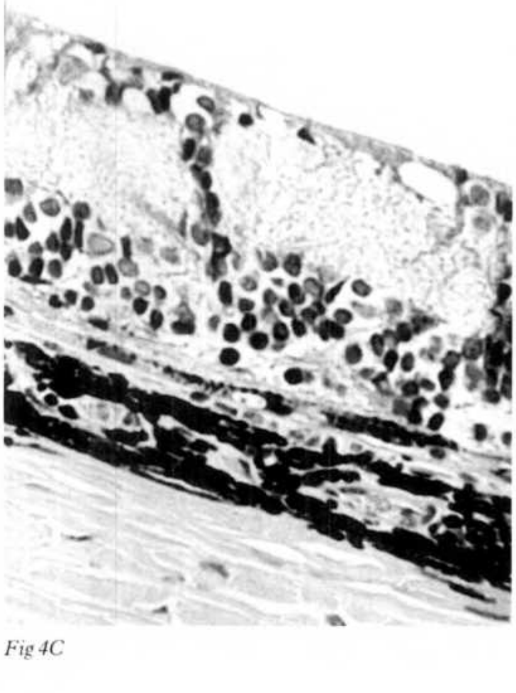

Fig 4C

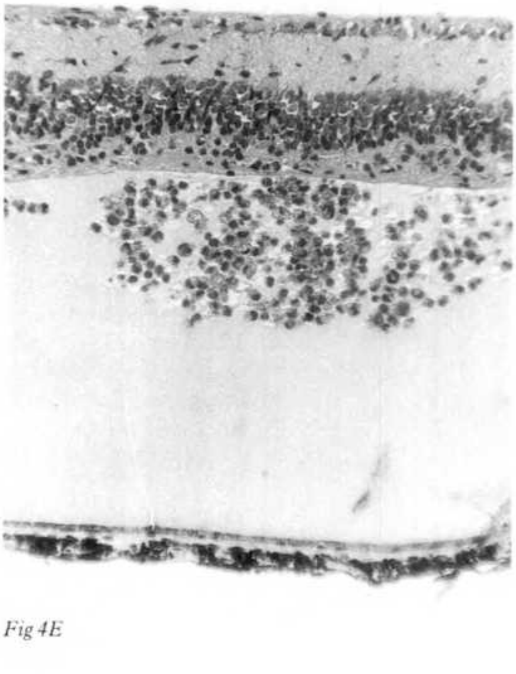

Fig 4E

debris accumulates between the photoreceptor layer and the RPE. Secondly, the interphotoreceptor matrix, which promotes retinal adhesion, is abnormal early in the course of the dystrophy.[20] Thirdly, both the outer and inner blood-retinal barriers become spontaneously leaky to plasma protein as early as 21 days of age.[21] In EAU these characteristics may allow the early accumulation of large amounts of subretinal fluid and subsequent detachment prior to the maximal influx of inflammatory cells, so enabling sufficient antibody to enter the subretinal space to produce a local Arthus reaction.

We did not find evidence of altered susceptibility to EAU with increasing age at sensitisation in the dystrophic rats though there was a tendency for EAU onset to be delayed in the older animals. There is known to be a reduced susceptibility to, and reduced severity of EAU in animals when photoreceptor outer segment turnover is reduced by disturbance of the normal day/night cycle. Thus, rearing in constant light[3] (which induces photoreceptor degeneration) or constant dark[22] (which moves S-antigen to the inner portions of the photoreceptor layer) will reduce the severity of EAU as well as reduce the amount of S-antigen in the photoreceptor outer segments. We find that constant dark rearing also delays the onset of EAU even in the high responder Lewis strain of rat (E Kasp and S Suleyman, unpublished data). These findings contrast with the exacerbation of disease severity brought on by constant dark, in the collagen induced arthritis model of autoimmune disease.[23]

In the present experiments, where RCS photoreceptor metabolism is disturbed because of the RPE abnormality, it is difficult to know whether the amount of S-antigen available to sensitised recirculatory T lymphocytes is reduced because of the reduced number of photoreceptors, or increased because of the disruption to the blood-retinal barrier. The tendency for an earlier onset, and shorter course of disease in the dystrophic strain, would be expected if there was a relatively greater access to a reduced amount of the target antigen.

Altered expression of EAU in the dystrophic strain might also arise from innate sensitisation to photoreceptor proteins, known to occur in both animals and humans with photoreceptor dystrophies. The RCS rat spontaneously develops IgM antibodies against photoreceptors in the early stages of dystrophy[24] and a small minority develop raised levels of antibody against S-antigen itself.[25 26] Raised levels of antibodies to photoreceptors have been detected in humans with retinitis pigmentosa (RP)[27 28] and the frequency of retinal-specific B cells (found after Epstein-Barr virus transformation) is higher than normal.[29] In RP patients T suppressor cells are reduced and there are slight increases in interleukin-2 receptor expression or peripheral lymphocytes and in HLA class II expression on RPE cells.[30-34] These changes, taken together, suggest that in the RCS rat as well as in retinitis pigmentosa the immune system is primed for autoimmune reactions against the retina, which may well contribute to the disease process. Antiretinal antibodies are also known to be associated with increased retinal vascular permeability in a variety of degenerative and inflammatory retinal disorders in man.[35]

In conclusion, this model of EAU demonstrates that a genetically determined biochemical dysfunction of target photoreceptor cells influences the clinical expression of retinal disease produced by peripheral autosensitisation to S-antigen. Further characterisation of the vascular and cellular events in early EAU in the RCS dystrophic rat may help to explain how these genetic factors could be operating at the molecular level.

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MINI REVIEW

Adjustable sutures in squint surgery

Despite widespread attention in the ophthalmic literature the use of adjustable sutures in squint surgery remains limited in the United Kingdom. This review examines the advantages of adjustable sutures, surgical indications, and operative techniques.

The concept of adjustable suture surgery was originally developed more than 50 years ago,[1] but it was not until the mid 1970s that the technique was popularised by Jampolsky[2] following work on squint surgery under topical anaesthesia with on table adjustment.[3] A one stage procedure with adjustment under general anaesthesia has been described,[4] but ocular alignment depends solely on examination of the corneal light reflexes. Furthermore, the resting position of the eyes is affected by general anaesthesia,[5] although attempts have been made to estimate the 'true' position and predict optimal postoperative alignment.[6,7] The majority of surgeons now opt for a two stage procedure; initial surgery being carried out under general anaesthesia, with postoperative adjustment under topical anaesthesia.

Indications

Adjustable sutures are intended primarily to facilitate more accurate ocular alignment and decrease the need for reoperation. In patients with fusion an optimal position within the fusional range can be selected, minimising postoperative diplopia and maximising the functional field of binocular single vision. In cosmetic procedures the most appropriate ocular alignment is more easily attained.

Indications for the use of adjustable sutures vary widely – 'a horizontal or vertical deviation requiring a weakening or strengthening procedure on a rectus muscle in a cooperative patient' being proposed by the more enthusiastic.[8] The advantages of an adjustable technique are most apparent when the results of conventional surgery are unpredictable, particularly as a result of tethering, scarring, or contracture of extraocular muscles. Specific indications include reoperations, large angle squints, thyroid eye disease, blow-out fractures, diplopia following retinal detachment surgery, paralytic squints, and combined horizontal and vertical muscle procedures. A possible limitation of the technique is that it is difficult to perform vertical transposition in conjunction with horizontal muscle surgery[9,10] although this is not universally accepted.[11]

Preoperative assessment

A full explanation of the procedure, including details of the adjustment, is mandatory. To gauge the suitability of a patient for adjustment, topical anaesthesia is applied and a cotton bud used to manipulate the bulbar conjunctiva.[12] Patients tolerating the 'cotton bud test' are likely to cooperate with adjustable surgery. In our experience this includes most adults and some children as young as 8 years.

The two stage approach

The anaesthetist should be aware that an adjustable procedure is planned so that the patient is as alert and cooperative as possible in the early postoperative period. Day case surgery is ideal, the operation being performed in the morning and adjustment carried out later the same day. Adjustment is possible with recession or resection, on horizontal or vertical muscles, on two muscles of the same eye and on both eyes in a bilateral procedure. Adjustment may be performed as soon as the patient is sufficiently alert to cooperate, usually within 6 hours of surgery. Waiting until the following morning is an acceptable alternative, but difficulty may be encountered as some muscles adhere firmly to the globe within 24 hours.

Limbal, Swan, and fornix incisions are practised. We prefer the limbal approach which has the advantage of good exposure, allows conjunctiva to be recessed in cases of restriction and is generally favoured in re-operations. Difficulty may be encountered when contact lenses are worn and required at the time of adjustment; in such cases a Swan or fornix incision is preferred.[12,13]

Two principal techniques of muscle reattachment have been described, the 'bow tie'[2] and the 'sliding knot'.[11] In the bow-tie technique the arms of the suture are passed through scleral tunnels starting near each end of the insertion and emerging 1·5 mm apart, advancing nearer to the limbus to facilitate adjustment.[11] A double throw is placed, with the muscle correctly positioned and secured with a loop. Fells passes both arms of the suture through the insertion twice, each time from posterior to anterior to form two 'Z' configurations.[12] The position is maintained by friction alone and only a loose bow need be tied. When a sliding knot is to be employed, the two arms of the suture are brought out parallel and close together at the insertion, and lassoed with a second piece of 6/0 vicryl. This is tied tightly around the muscle suture and snugged down to the insertion. If the muscle is then pulled anteriorly as far as the insertion, the distance between sliding knot and insertion can be measured, and will be equal to the amount of recession performed. This technique allows accurate estimation of the final muscle position following adjustment, but tends to leave a larger knot. In a final variation, Campos leaves the two ends of the vicryl suture completely untied allowing the muscle to hang back freely, thereby committing himself to adjustment.[14] Subsequent adjustment in all cases is eased by the ability to fix the globe, which is best done with the aid of a 'bucket handle' suture, such as 6/0 vicryl (Polyglactin 910), placed in the sclera at or anterior to the insertion.

A key factor in the success of adjustable sutures has been the use of the double armed 6/0 vicryl suture on spatulate needles. Despite its gauge, 6/0 vicryl has remarkable tensile strength and little tendency to snag. Identification at the time of adjustment is eased by using a dyed suture and the handling characteristics may be improved by coating with bone wax. A tendency for 'tissue drag' to occur between conjunctiva, Tenon's capsule, and muscle can be reduced by lubrication with Healon,[15] although this is rarely necessary.

Some surgeons deliberately over-recess at operation as it is easier to advance rather than recess a muscle at the time of adjustment. Such a strategy inevitably increases the number of cases requiring adjustment, and it is our policy to recess the exact amount required. When resecting a muscle, it is usual to resect slightly more than the estimated amount, and then leave the muscle recessed to allow adjustments either way. Excess resection, however, would increase both the risk of restriction and the documented incidence of muscle slippage,[8 16] making adjustable resection in general a far less popular option.

Several strategies have been proposed for dealing with the conjunctival flap following a limbal incision. In the 1970s, problems were encountered with irritation from the non-absorbable sutures used at that time[17] but this is much less apparent with vicryl. It should be remembered that some squint operations are cosmetic, and that the handling and repair of conjunctiva is an important part of the operation. Jampolsky originally advocated leaving the knot exposed on bare sclera, and excising it 2 to 3 weeks postoperatively.[11] We routinely close conjunctiva, with a loosely recessed flap allowing access to the bow. Alternatively, sutures may be placed through the corners of the flap and corresponding limbal conjunctiva in the first stage of the procedure, with either one[18] or both[19] corners being left mobile on loosely tied loops to allow access for adjustment. Finally, conjunctiva may be closed as an entirely secondary procedure at the time of adjustment.[4] When conjunctiva is closed, it is important to ensure that no restriction of ocular movement is induced.

After the first stage the suture ends are tucked into the conjunctival fornix or taped to the skin. Antibiotic drops are instilled and the eye padded. The pad is removed at least 30 minutes before adjustment (as occlusion is known to alter ocular muscle balance[20]) and topical anaesthetic drops instilled. The correct refraction must be worn by the patient during assessment, the surgeon having ensured that no prismatic correction has been incorporated. Adjustment is a 'semi-sterile' procedure performed at the bedside or in the clinic, the requirements being adequate lighting and a distant fixation target. Correcting any underaction is straightforward as an over-recessed muscle is easily advanced. An overacting muscle is further recessed by slackening off the suture, and then fixing the globe by means of the bucket handle while the patient attempts to look in the direction of action of the muscle. Several adjustments may be necessary to achieve the correct alignment, at which time the bow tie is converted to a knot, or the limbs of the sliding knot and muscle sutures cut short.

Long term success requires an understanding of the optimal postoperative alignment. Early drift of several dioptres is known to occur[4 9] and may be predicted. A tendency has been noted for initial over-correction of a horizontal deviation to reverse, prompting some authors to adjust only those patients with significant under-correction.[21] Rosen *et al*, studying post adjustment drift in the first 12 months, observed a swing towards under-correction of horizontal deviations and over-correction of vertical deviations, the former occurring more rapidly in cosmetic than in functional groups.[22] Wright has observed a greater final under-correction of the angle of squint following bilateral recessions, compared with unilateral recess-resect procedures, for esotropia.[23]

Complications

Complications are rare. Nausea may be induced by adjustment, but careful handling of the globe minimises both this and the risk of bradycardia. Breakage of the suture is very uncommon, as is the inability to achieve a satisfactory alignment. Parks has questioned the use of any form of 'hang loose' suture, stressing both the tendency for the muscle width to collapse and the potential uncertainty of the final position of attachment of muscle to sclera.[9] Inferior rectus is prone to slip postoperatively as a consequence of its attachments to Lockwood's ligament and inferior oblique: Kushner has advocated the use of a non-absorbable adjustable suture in this situation.[10]

Results

The recognition of any new surgical technique as successful is best made with reference to prospective studies comparing the newly proposed procedure with a previously accepted technique. Such information is not available, but results have been published and should be considered carefully. The absolute criterion for failure in squint surgery is the need to reoperate. Two retrospective studies have shown a decrease in the reoperation rate comparing adjustable sutures with conventional surgery.[24 25] Other authors have examined their reoperation rates following adjustable techniques, quoting figures of 5–8%,[17 18 26] Alternatively, considering 'success' to be a final deviation of less than 10 prism dioptres horizontally or 5 prism dioptres vertically, Kraft and Jacobson typify published results with a success rate of 85% for primary procedures and 78% for reoperations.[18] Their 'adjustment rate' of 40% is similar to that of other studies.[17 24–26]

Conclusion

Success in ocular motility surgery depends on many factors. The adjustable suture technique is designed to minimise variability in one of these factors, namely the idiosyncratic response of an individual patient to a stereotyped surgical procedure. Adjustable sutures have been employed across the whole spectrum of squint surgery, from the cosmetic correction of a divergent blind eye to the functionally complex Harada-Ito procedure.[27 28] Each surgeon must base his practice on his own results, the particular needs of the individual patient and the time and resources available. The techniques outlined above are simple to perform and take up no more theatre time than conventional squint surgery. Although adjustable suture surgery is not at present regarded as routine, the simple objective of avoiding large postoperative ocular misalignment in unpredictable cases should endear this procedure to all squint surgeons.

ROBERT J MORRIS
ANDREW J LUFF

Southampton Eye Hospital,
Wilton Avenue,
Southampton SO9 4XW

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British Journal of Ophthalmology 1992; **76**: 563–566

CASE REPORTS

Congenital adenoma of the iris and ciliary body: light and electron microscopic observations

Ian G Rennie, M Andrew Parsons, Colin A L Palmer

Abstract

A 23-year-old man had a lesion in the right inferior iris which appeared to have enlarged since it was first seen when the patient was aged 5 years. The lesion was excised by a partial iridocyclectomy. Histopathologically the neoplasm was composed of both pigmented and non-pigmented cells. Pseudoacini, containing acid mucopolysaccharides, were present throughout the tumour matrix. Electron microscopically the non-pigmented cells were found to possess a convoluted plasmalemma, abundant rough endoplasmic reticulum, and numerous desmosomes and gap junctions. The pigmented cells contained large, round, mature melanosomes, occasional premelanosomes, and desmosomes, which resembled the posterior pigment epithelium of the iris. The intercellular matrix contained fine collagen fibrils resembling vitreous. We believe that this neoplasm represents a congenital adenoma of the ciliary body and iris.

(*Br J Ophthalmol* 1992; **76**: 563–566)

True neoplasms arising from the non-pigmented ciliary epithelium are extremely rare.[1-13] Zimmerman[1] has proposed that they are classified into congenital and acquired lesions, based on their histological appearances. Congenital lesions arise from the primitive medullary epithelium and include glioneuromas and medulloepitheliomas. Acquired neoplasms and pseudoneoplasms of the non-pigmented ciliary epithelium usually occur in adulthood; they arise from the fully differentiated ciliary epithelium and include: psuedoadenomatous hyperplasia (reactive and senile), adenomas, and adenocarcinomas.[14] Lesions arising from the pigmented epithelium are equally uncommon and include adenomas[15-18] and adenocarcinomas.[19 20] Adenomas of the iris pigment epithelium have also been described.[21 23] We report a congenital adenoma of the ciliary body and iris, which contained well differentiated pigmented and non-pigmented elements.

Case report

The patient, a 23-year-old man, first presented to the eye department aged 5½ years with a hyphaema in the right eye following an injury with a cardboard toy. The patient was admitted for bed rest, and upon resolution of the hyphaema, was noted to have a greyish mass in the iris inferiorly. The patient's parents commented that there had always been a 'mark' on the iris, and a photograph taken when the patient was 26 months old confirmed this. Staff opinions were sought and it was the consensus that the lesion was benign, possibly a leiomyoma, and should best be managed by periodic observation.

When the patient was reviewed in January 1987 the lesion appeared to have enlarged slightly and further staff opinions were sought. On examination the uncorrected visual acuities were 20/15 in both eyes. Slit-lamp biomicroscopy revealed a nodular, variably pigmented, wedge shaped lesion involving the inferior iris and angle (Fig 1). The iris stroma was deficient overlying the lesion and an adjacent cortical lens opacity was present. Prominent dilated blood vessels were present on the surface of the lesion and in the adjacent iris stroma. Dilated episcleral (sentinel) vessels were noted overlying the adjacent sclera. The remainder of the ocular examination was normal. In view of the possible enlargement and uncertain diagnosis, the lesion was locally excised under general anaesthesia. Postoperative progress was uneventful and the visual acuity 3 months after surgery was correctable with a contact lens to 20/20. The patient had been followed for 3 years without any signs of recurrence.

Pathological examination

LIGHT MICROSCOPY

Microscopically the neoplasm was composed of

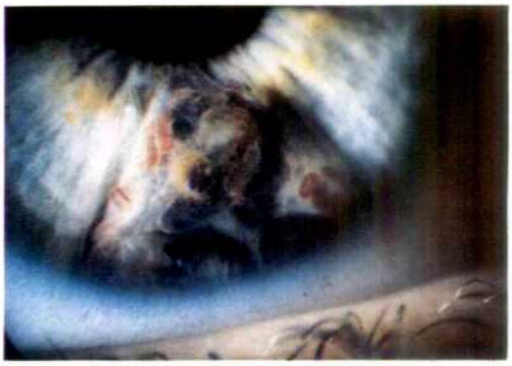

Figure 1 Clinical appearance of the right eye showing a nodular, variably pigmented tumour arising in the inferior iris.

University of Sheffield, Department of Ophthalmology
I G Rennie
C A L Palmer

Department of Pathology
M A Parsons

Correspondence to:
Mr I G Rennie, Department of Ophthalmology, Royal Hallamshire Hospital, Glossop Road, Sheffield S10 2JF.

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pigmented and non-pigmented elements (Fig 2). The non-pigmented cells predominated and formed both solid and cystic areas, the latter containing a mucinous material which stained positively for acid mucopolysaccarides with Alcian blue. The non-pigmented cells were eosinophilic, with poorly defined cell borders and counterstained small, mildly hyperchromatic irregular nuclei. Occasional cells contained larger nuclei with a small, but distinct, nucleolus. Mitotic figures were not observed. The pigmented cells, which tended to form strands running through the tumour matrix, had a densely pigmented cytoplasm which obscured nuclear detail. Normal iris and ciliary processes were present at opposite ends of the specimen. Ciliary epithelium containing both pigmented and non-pigmented elements extended over the surface of the lesion towards the iris where, after a transitional zone, the epithelium acquired the characteristics of the posterior pigment epithelium of the iris (Fig 3).

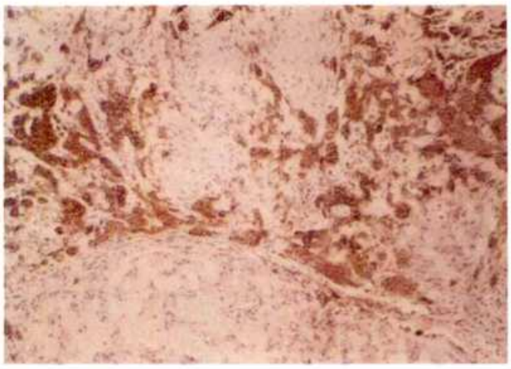

Figure 2 Photomicrograph of the tumour showing the pigmented and non-pigmented cellular elements. Pseudoacini can be seen on the inferior edge of the section (haematoxylin and eosin, magnification ×130).

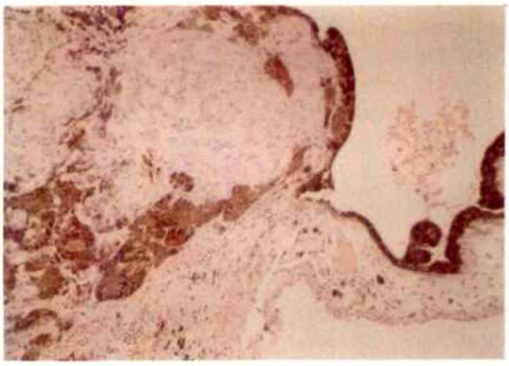

Figure 3 Photomicrograph showing the transition between tumour and normal iris. Normal posterior pigment epithelium can be seen extending over the tumour surface (haematoxylin and eosin, magnification ×66).

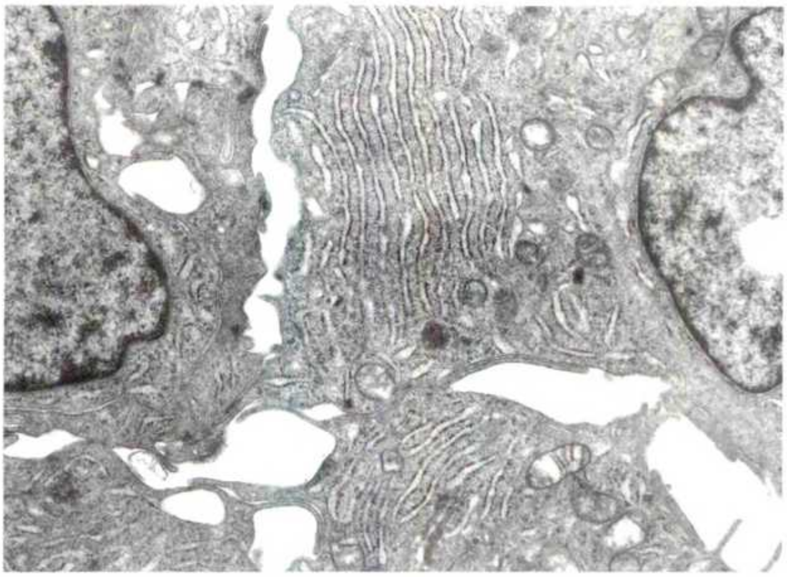

Figure 4 Non-pigmented tumour cells, containing plentiful rough endoplasmic reticulum and numerous mitochondria (magnification ×7375).

ELECTRON MICROSCOPY

Glutaraldehyde fixed material was submitted for electron microscopy. Both pigmented and non-pigmented cells were readily identified. The non-pigmented cells contained numerous mitochondria and plentiful rough endoplasmic reticulum (Fig 4). In some areas light and dark cells were present. The cell nuclei were elongated with mildly convoluted nuclear membranes, and occasionally contained a small, single nucleolus. The cells had a deeply invaginated plasmalemma, with frequent interdigitations and tight junctions between adjacent cells (Fig 5).

The pigmented cells contained large, round, mature melanosomes, occasional pre-melanosomes and very occasional mitochondria (Fig 6). Fine fibrils were not observed in these cells which structurally resembled those of the iris posterior pigment epithelium.

Both pigmented and non-pigmented cells were joined by well developed desmosomes and tight junctions (Fig 6). Numerous extracellular spaces (pseudoacini) containing fine collagen fibrils were present throughout the tumour. The cells lining the extracellular spaces had a prominent, often reduplicated, basal lamina (Fig 7).

Discussion

Acquired neoplasms of the non-pigmented ciliary epithelium usually occur in adults and arise from fully differentiated tissue, in contrast to congenital neoplasms, which are derived from undifferentiated medullary epithelium.[1,14] The most common acquired tumour-like lesion is the coronal adenoma (senile hyperplasia, Fuchs' adenoma) which is a pseudoadenomatous hyperplasia of the pars ciliaris. These lesions are rarely diagnosed clinically but are commonly observed in surgical or autopsy specimens.[24,25] Typically they are small white, often multiple, globular tumours, arising from the pars plicata. They rarely occur before the age of 50 years, but are found with increasing frequency thereafter.[24,25] Trauma or inflammation may induce a pseudoadenomatous reactive hyperplasia of the ciliary epithelium, which may contain both pigmented and non-pigmented elements.[14] In contrast, true neoplasms of the ciliary neuroepithelium are extremely rare and most reports are limited to single cases.[5-20] Adenomas and adenocarcinomas may arise from either the pigmented[15-20] or non-pigmented[5-13] ciliary epithelium.

Our case differs from most of those previously reported in several respects: the lesion was first noted in infancy and may well have been congenital; it was observed for more than 17 years

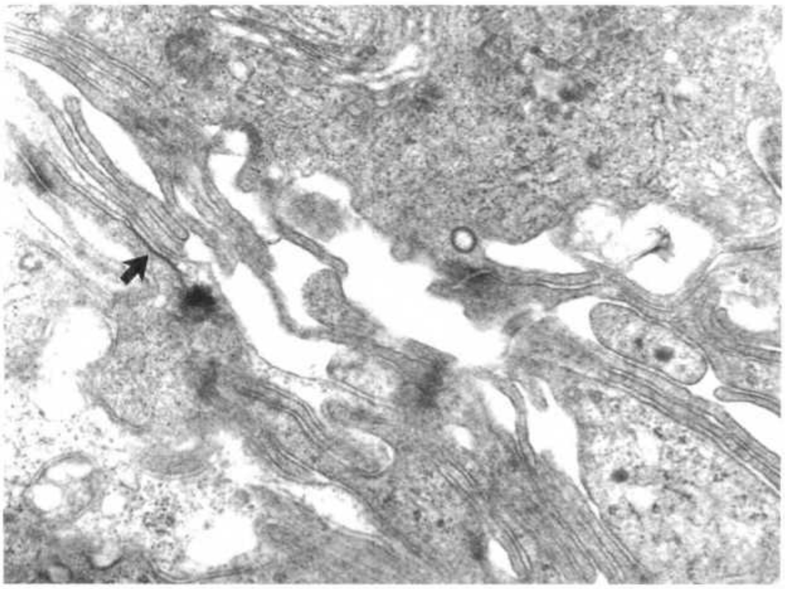

Figure 5 There are complex interdigitating processes and tight junctions (arrow) between adjacent non-pigmented tumour cells (magnification × 11 800).

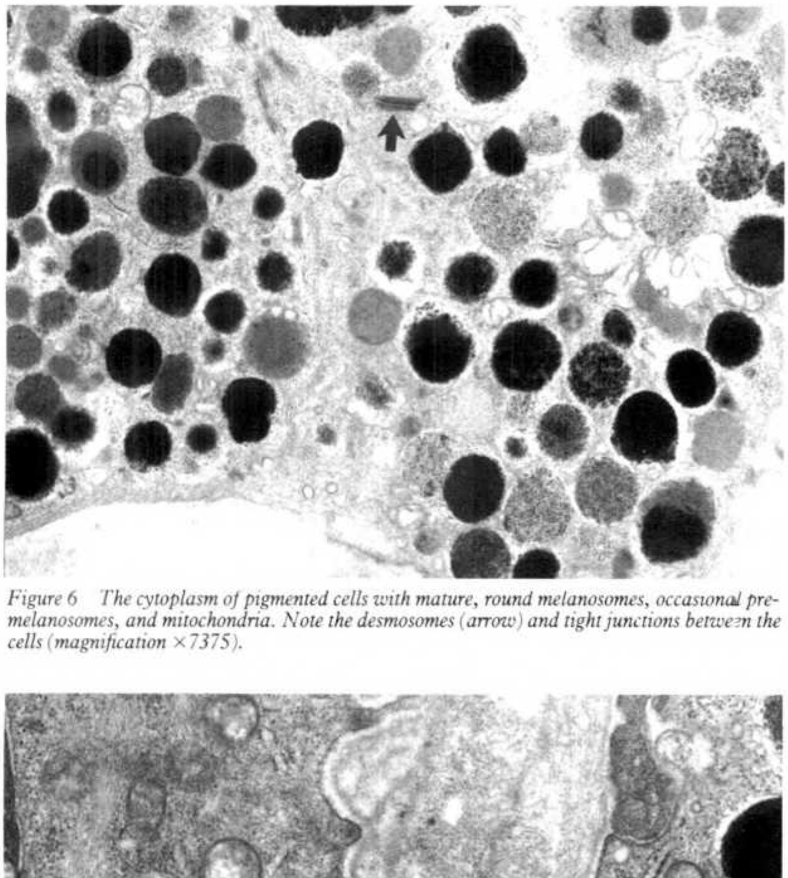

Figure 6 The cytoplasm of pigmented cells with mature, round melanosomes, occasional premelanosomes, and mitochondria. Note the desmosomes (arrow) and tight junctions between the cells (magnification × 7375).

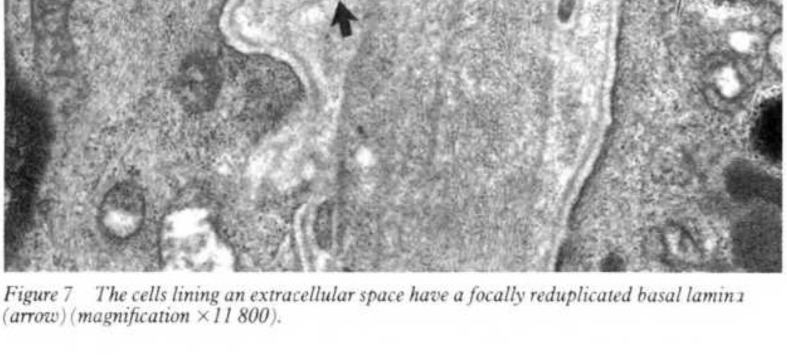

Figure 7 The cells lining an extracellular space have a focally reduplicated basal lamina (arrow) (magnification × 11 800).

prior to treatment, and contained both pigmented and non-pigmented cellular elements. If the lesion were present at birth, then it represents an example of a congenital neoplasm which was composed of fully differentiated cellular elements. In this respect the tumour reported herein has similarities with the lesion described as a 'hamartomatous adenoma' of the non-pigmented ciliary epithelium reported by Patrinely *et al*[26]; they reported a case of a slowly enlarging mass arising at the site of a coloboma of the iris and ciliary body of a 2½ year old infant. Because of its association with a coloboma they felt the tumour represented a hamartoma rather than a conventional adenoma of the ciliary body. It is interesting to note that our neoplasm was also situated inferiorly and was deficient of iris stroma overlying its surface. Ultrastructurally there are similarities between the two lesions; in both cases the lesions contained dark and light non-pigmented cells, which exhibited well-developed endoplasmic reticulum, complex plasmalemmal infolding, desmosomes, and tight junctions. In addition, multilaminar basal lamina and spaces containing fine fibrillar material, structurally similar to vitreous, were observed in both lesions. All of these features would suggest that the non-pigmented cells are similar to those of the fully differentiated non-pigmented ciliary epithelium. Our tumour differed from the described by Patrinely *et al*[26] in that it contained numerous pigmented cells which are structurally similar to those of the normal iris posterior pigment epithelium. The presence of cells which resemble both non-pigmented ciliary epithelium and iris posterior pigment epithelium would suggest that this tumour may have developed during the differentiation of the primitive medullary epithelium in utero, and in this respect it conforms with the classification proposed by Zimmerman.[1] It differs however, from other congenital lesions (glioneuromas and medulloepitheliomas) in that it is composed entirely of fully differentiated cells; this suggests that the tumour, initiated in utero in the primitive medullary epithelium, may have progressed by division of the two resulting fully differentiated cell types.

The benign nature the lesion is suggested by its relative indolence over a 17 year period. Eventually it was felt that some growth had taken place, although it is possible that this may have been due to an increase in the intercellular matrix (auxetic growth) rather than a proliferation of the cellular elements (multiplicactive growth).

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British Journal of Ophthalmology 1992; **76**: 567–568

Idiopathic choriovitreal membrane – a case report

Stephen H Sinclair, Brian D Salmenson

Abstract

A case of a macular idiopathic choriovitreal membrane is described which developed in a diabetic man. On initial examination the patient was found to have a pigment epithelial detachment with a choroidal neovascular membrane (CNVM) in the right eye. Two months after the first visit the CNVM was seen to have penetrated the retina and presented as a choriovitreal membrane. Panretinal photocoagulation was applied after which the choriovitreal membrane demonstrated fibrotic involution. This case is unusual in that the choriovitreal membrane developed in the absence of a choroidal or retinal pigment epithelial disease process that may be associated with a CNVM as well as in the absence of previous macular laser treatment.

(*Br J Ophthalmol* 1992; **76**: 567–568)

Choroidal neovascular membranes (CNVMs) are usually confined to the subpigment epithelial or subretinal space unless the retina has been traumatised (for example, by laser photocoagulation), in which case they have been reported to produce chorioretinal or choriovitreal neovascularisation. We report a case of an idiopathic CNVM which penetrated an intact retina to produce a choriovitreal fibrovascular membrane.

Case report

A 69-year-old insulin-dependent diabetic man presented with decreased vision in both eyes due to vitreous haemorrhage from disc and peripheral retinal neovascularisation. The visual acuity in the right eye was counting fingers at 2 metres and in the left eye 6/12. The anterior segments were normal except for nuclear sclerosis of both lenses. On biomicroscopy of the posterior segments, a pigment epithelial detachment with a CNVM was noted beneath the fovea of the right eye and was documented by fluorescein angiography (Fig 1A and B). No drusen, retinal pigment epithelial changes, or macular retinal exudates were observed in either fundus. Proliferative diabetic retinopathy was present in both eyes with disc and peripheral retinal neovascularisation as well as vitreous haemorrhage noted in the right eye (Fig 1A). Panretinal photocoagulation (PRP) was begun in both eyes because of the high risk characteristics of proliferative diabetic retinopathy. During the course of the laser the CNVM in the right eye was observed by stereoscopic biomicroscopy to have broken through the surface of the retina within the fovea and to grow as an epiretinal, fibrovascular membrane internal to the surface of the retina. The posterior hyaloid had not separated from the surface of the macula (Fig 2). The visual acuity in the right eye had decreased to hand movements at 1 metre. On follow-up examination a few weeks after completion of panretinal photocoagulation to the right eye, the macular epiretinal fibrovascular membrane demonstrated progressive fibrosis with adherence to the posterior hyaloid and elevation from the surface of the retina producing a tractional retinal detachment (Fig 3). Vitreous surgery was attempted to reduce the progressing retinal detachment. At the time of delamination of the epiretinal membrane, a fibrous stalk was noted to extend from the subretinal space through the foveal region to the epiretinal component attached to the posterior hyaloid.

Discussion

Numerous causes of CNVMs are recognised, all of which are known or thought to occur with a defect in Bruch's membrane. When no cause can be determined clinically they may be labelled as idiopathic. In the case described here a subretinal CNVM developed in an elderly diabetic man without drusen, without previous laser therapy or evidence of optic disc drusen, or a retinal

Department of Ophthalmology, Hahnemann University Medical School, Philadelphia, PA, USA
S H Sinclair
B D Salmenson

Correspondence to:
Stephen H Sinclair, MD,
Department of Ophthalmology, Hahnemann University Medical School,
Broad and Vine Streets,
Philadelphia, PA 19102, USA.

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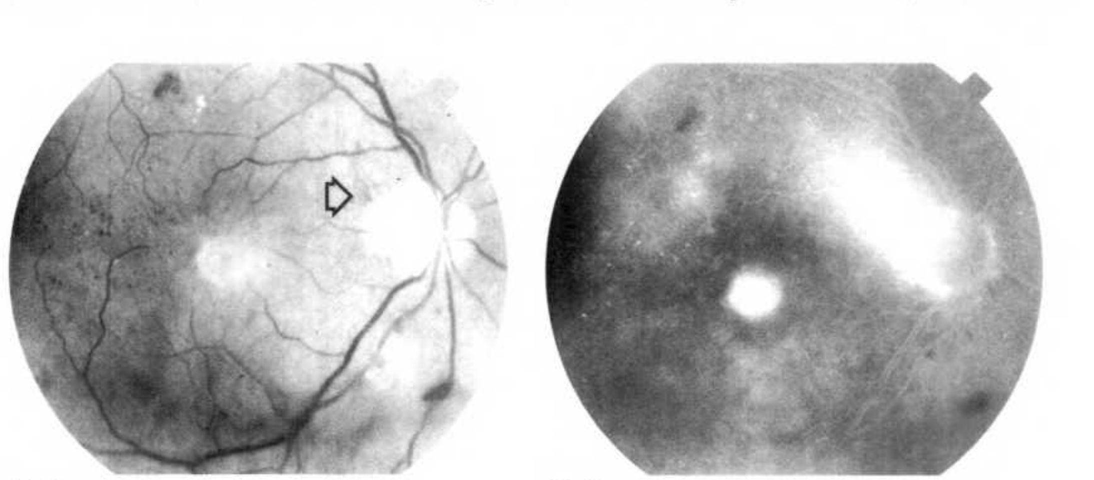

Fig 1A

Fig 1B

Figure 1 (A) A red-free photograph of the right fundus at first presentation demonstrating a subfoveal choroidal neovascular membrane and diabetic retinopathy with epipapillary neovascularisation (arrow) and vitreous haemorrhage. (B) Fluorescein angiogram of the right fundus at first presentation demonstrating the foveal choroidal neovascular membrane and leakage from the epipapillary neovascularisation.

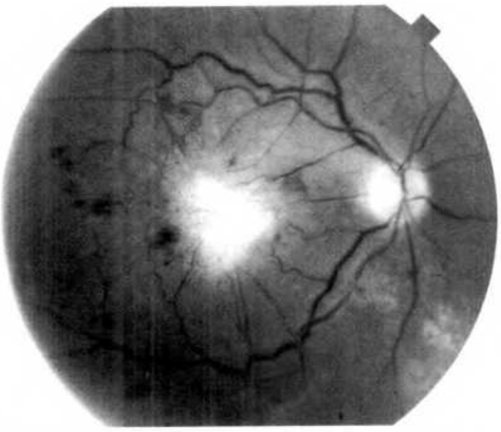

Figure 2 Red-free fundus photograph of the right eye demonstrating a fibrovascular membrane on the inner surface of the retina overlying the macular retinal vessels.

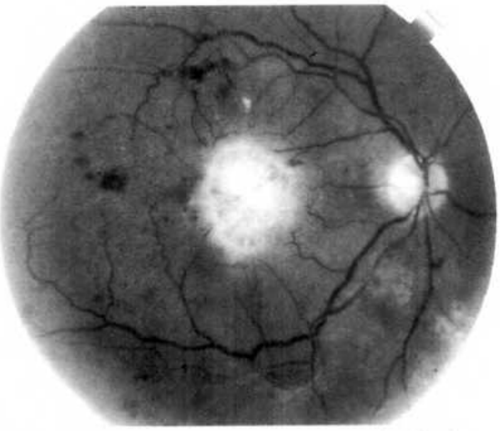

Figure 3 Fundus photograph demonstrating the fibrotic lesion in the right macula following panretinal photocoagulation.

pigment epithelial disease process that has been associated with CNVM. The early appearance of the CNVM confined to the subretinal space appeared similar to those described by Yeo *et al*,[1] where subretinal fibrosis developed in diabetic macular oedema associated with large lipid deposits. In only one of their cases was the subretinal fibrosis secondary to a CNVM and in all of their cases the nodular subretinal fibrosis followed extensive macular exudate, which was not observed here. Within 2 months the CNVM observed in our patient had grown through the retina and appeared as an epiretinal fibrovascular membrane with adherence to the posterior vitreous hyaloid and on surgery was noted to extend from the subretinal space as choriovitreal neovascularisation.

Subretinal neovascular membranes of choroidal vascular origin have been reported following heavy photocoagulation for diabetic macular oedema, proliferative diabetic retinopathy, proliferative sickle retinopathy, central serous chorioretinopathy, presumed ocular histoplasmosis syndrome, choroidal haemangiomas, and choroidal melanomas. Extension of the CNVM to form choriovitreal neovascular membranes has been described following intense photocoagulation for proliferative sickle cell reinopathy,[2] proliferative diabetic retinopathy,[3] sarcoidosis,[4] and malignant melanoma.[5] To our knowledge, however, this is the first report of a CNVM spontaneously growing through an intact retina to produce choriovitreal neovascularisation.

CNVMs following intense photocoagulation are thought to develop through a disruption in Bruch's membrane and have been reproduced in rhesus monkeys. In the animal model infiltration of the retina occurred if there was coexistent inner retinal ischaemia which was the case in our patient's eye. Inner retinal ischaemia may also be associated with changes at the level of the retinal pigment epithelium (RPE).[6] The diabetic eye may demonstrate thickening of the RPE basement membrane and narrowing of the choriocapillaris lumen,[7,8] all of which may have contributed to the formation of the CNVM and promoted its penetration of the thin retina at the foveola where it arose.

Choriovitreal neovascularisation has in general been thought not to respond to panretinal photocoagulation. Augsburger *et al*[4] however observed regression of such neovascularisation in two patients following scatter photocoagulation of areas of retinal ischaemia peripheral to an area of choriovitreal neovascularisation after treatment for malignant melanoma. Although the CVNM in our case did demonstrate some fibrosis prior to photocoagulation for the proliferative diabetic retinopathy, the involution of the membrane appeared to have been accelerated by the photocoagulation. Choriovitreal neovascularisation was successfully treated with direct argon and/or xenon photocoagulation in two of four eyes treated by Dizon-Moore.[2] Direct treatment was not considered in our case owning to the location of the membrane. Because of the progressive tractional retinal detachment, vitrectomy with en bloc membrane delamination was attempted and was successful at releasing the detachment and improving the central scotoma and vision, though the macula remained puckered due to the subretinal fibrosis.

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Ciliary block (malignant) glaucoma after cataract extraction with lens implant treated with YAG laser capsulotomy and anterior hyaloidotomy

A Halkias, D M Magauran, M Joyce

Abstract

An 84-year-old woman developed high intraocular pressure with a shallow anterior chamber 2 months after an extracapsular cataract extraction with posterior chamber lens implant. The condition did not respond to peripheral iridectomy and removal of the implant but was treated successfully with YAG laser capsulotomy and anterior hyaloidotomy.

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Malignant glaucoma is a rare complication of intraocular surgery. It usually follows glaucoma surgery but has also been reported, among others, after modern cataract surgery.[1 2]

Case report

The patient was an 84-year-old woman who had ophthalmic history of bilateral cataracts, bilateral age-related macular degeneration, and an old corneal scar in her left eye. She had a left intracapsular cataract extraction with anterior chamber lens implant in 1985.

Postoperatively she developed what was considered to be pupil block glaucoma with a moderate rise of intraocular pressure (around 30 mm Hg) and a thickening of the anterior hyaloid face which reduced the visual acuity (to 6/60 corrected). Both conditions were successfully treated with YAG laser iridotomy and anterior hyaloidotomy. She was a known hypermetrope with axial lengths of right eye 21·17 mm and left eye 20·60 mm.

She had a right extracapsular cataract extraction with a posterior chamber lens implant in 1990. The surgery was complicated by miosis which, in conjunction with the old corneal scar, made visualisation difficult. A small amount of soft lens matter was left in the 9 o'clock position. The posterior capsule was intact.

Postoperatively the lens matter was absorbed and the visual acuity improved to only 6/24 corrected because of her macular degeneration. Two months postoperatively she developed severe pain in her right eye and was admitted as an emergency.

Her visual acuity was reduced to hand movements. There was perilimbal injection, corneal epithelial oedema, the anterior chamber was shallow especially at 9 o'clock, and the intraocular pressure was 66 mm Hg. She was treated with intensive pilocarpine drops and systemic acetazolamide; the pressure was then 35 mm Hg. However the anterior chamber remained shallow. She underwent two surgical iridectomies and the anterior chamber was reformed with Healon. These measures were unsuccessful and the anterior chamber remained shallow. The intraocular pressure was 40 mm Hg. The implant was removed and the anterior chamber was reformed with Healon. The posterior capsule was intact.

The anterior chamber became shallow again and the intraocular pressure was raised. A diagnosis of malignant glaucoma was made. Atropine, phenylephrine and steroid drops were given with systemic acetazolamide. The anterior chamber deepened for the first time, the intraocular pressure was still high, in the mid thirties mm Hg.

At this stage it was decided to establish a communication between the fluid which presumably had accumulated in the vitreous and the anterior segment. The YAG laser was used to make a central opening in the posterior capsule and disrupt the anterior hyaloid face. This resulted in an immediate deepening of the anterior chamber and a drop in the intraocular pressure which returned to normal over the next 48 hours. Her medication was gradually discontinued.

The visual acuity is 6/24 corrected, the anterior chamber is deep, and the intraocular pressure remains normal 10 months later.

Discussion

Malignant glaucoma is characterised by a shallow anterior chamber with persistently high intraocular pressure in spite of the presence of a patent iridectomy.[3 4 5]

The pathogenesis involves misdirection of aqueous which becomes trapped within or behind the vitreous. It is still unclear what the initiating event is but it is thought to be related to the particular anatomy of the eye (small eyes) combined with changes such as angle closure, inflammation of the ciliary body which pushes the iris-lens diaphragm forward, blocks the normal pathway of the aqueous, and also possibly causes adhesions of ciliary processes to the vitreous with resultant redirection of the aqueous flow towards the vitreal cavity. There it becomes entrapped, causes bulging of the vitreous, and the initiation of a vicious circle.[3 4]

In our case we hypothesise that the soft lens matter which remained after the extracapsular procedure caused an inflammatory reaction of the ciliary body with resultant adhesions of the processes to the vitreous and misdirection of aqueous in a predisposed eye. Alternatively, the operative trauma may have resulted in separation of the vitreous base from the pars plana. Misdirected aqueous then could have entered the

Department of Ophthalmology, Gloucestershire Royal Hospital
A Halkias
D M Magauran
M Joyce

Correspondence to:
Dr A Halkias, West Suffolk Hospital, Bury St Edmunds, Suffolk IP33 2QZ.

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vitreous body. It is known that if one eye suffers an attack of malignant glaucoma the fellow eye is also at risk. In our case we believe that the left eye suffered a 'mild attack' (unrecognised at the time since the intraocular pressure was not impressively high) and that the YAG laser treated the condition, probably not only by the creation of the peripheral iridotomy but also by the rupture of the anterior hyaloid face (which was done for optical reasons – that is, thickening).

The role of the anterior hyaloid as a barrier in this condition is recognised. The medical treatment of malignant glaucoma has been described some years ago.[4] Surgical treatment includes removal of the lens, posterior sclerotomy, incision of the vitreous, and vitrectomy. The incision of the anterior vitreous can be successfully performed by a YAG laser capsulotomy-hyaloidotomy in aphakic and pseudophakic eyes[6 7] and saves the patient another surgical intervention, as our case demonstrates.

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Late onset Leber's optic neuropathy: a case confused with ischaemic optic neuropathy

F-X Borruat, W T Green, E M Graham, M G Sweeney, J A Morgan-Hughes, M D Sanders

The National Hospital for Neurology and Neurosurgery, Neuro-Ophthamology Unit, Queen Square, London WC1 3BG
F-X Borruat
W T Green
E M Graham
M G Sweeney
J A Morgan-Hughes
M D Sanders

Correspondence to:
Dr F-X Borruat, The National Hospital for Neurology and Neurosurgery, Neuro-Ophthalmology Unit, Queen Square, London WC1 3BG.

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Abstract

A case is reported of a 63-year-old man with progressive central visual loss in one eye followed 11 months later by involvement of the fellow eye. A diagnosis of chronic ischaemic optic neuropathy was considered. However, despite a negative family history, the absence of electrocardiographic abnormalities, and minimal fundus changes a diagnosis of Leber's optic neuropathy was made on the basis of magnetic resonance imaging findings and the mitochondrial DNA mutation at base pair 11778.

(*Br J Ophthalmol* 1992; **76**: 571–573)

Central visual loss in one eye followed within a year by central visual loss in the fellow eye can present diagnostic difficulties in patients with a normal fundus examination. Exclusion of compression, infiltration, and a negative family history may lead to the diagnosis of ischaemic optic neuropathy (ION) in the elderly patient. In view of the therapeutic options an accurate diagnosis is important. We report the case of a 63-year-old patient without any family history of visual loss who presented with sequential bilateral optic neuropathy and a mitochondrial DNA mutation associated with Leber's optic neuropathy (LON). The diagnosis was supported by magnetic resonance imaging (MRI) and electrophysiological findings.

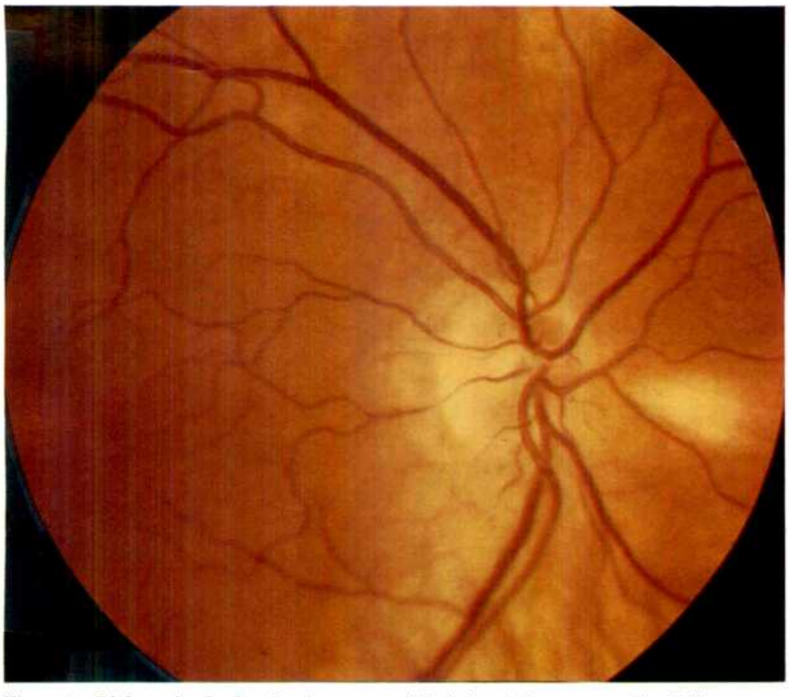

Figure 1 Right ocular fundus showing a normal sized disc without a cup with visible inferotemporal peripapillary capillaries. Medullated nerve fibres are seen nasally.

Case report

In May 1990 a previously healthy 63-year-old man noticed left visual loss accompanied by a constant, dull, left sided orbital pain. Visual function of the right eye was normal. There was no family history of visual disturbance and except for being an ex-smoker (up to 20 cigarettes a day) no vascular risk factors were found. His medical history was unremarkable. After investigations the diagnosis of left non-arteritic ION was made. Treatment with aspirin 300 mg/day was started. Left visual loss increased over several weeks and the left orbital pain remained for 3 months. In January 1991 the patient was referred to the National Hospital for Neurology and Neurosurgery. Right visual function was normal with distance visual acuity of 6/4, normal red-green colour vision (17/17 Ishihara plates), normal slit-lamp examination, and normal intraocular pressure (14 mm Hg). Right fundus examination was normal except for the presence of peripapillary nasal myelinated fibres. Left visual function was reduced to counting fingers at 1 foot, absent colour vision (Ishihara plates 0/17), and there was a left pupillary afferent defect. Left fundus examination revealed optic disc pallor.

Extraocular movements were normal. Goldmann perimetry was normal on the right but showed a central scotoma on the left side. Full-field pattern reversal visual evoked potentials (VEP) showed a normal latency (111 ms) and amplitude (7·6 μV) from the right eye and no detectable response from left eye. General examination was normal and laboratory investigations were unhelpful, including syphilis serology, CSF analysis, and computed tomography scan of head and orbits. These normal results supported the initial diagnosis of non-arteritic ION.

In April 1991, 11 months after the left visual loss, the patient noted difficulty identifying distant objects and, 7 days later, difficulty reading the newspaper. On the 16 April 1991 visual acuity in the right eye was decreased to 6/9 and N8, and red-green colour vision was also impaired (5/14 Ishihara). Goldmann perimetry was normal but tangent screen testing consistently demonstrated an inferior altitudinal loss to a small red target (3/1000 red). Fundus examination showed a normal sized disc with mild nasal swelling and visible inferotemporal capillaries (Fig 1). Fluorescein angiography showed no leakage (Fig 2). VEPs to pattern reversal showed a mild delay (120 ms) and reduced amplitude (3 μV) potential from the right eye and no potential from left eye stimulation. Orbital MRI scanning (short time inversion recovery (STIR) sequence) revealed the presence of a high signal within the left optic nerve but no abnormal signal from the

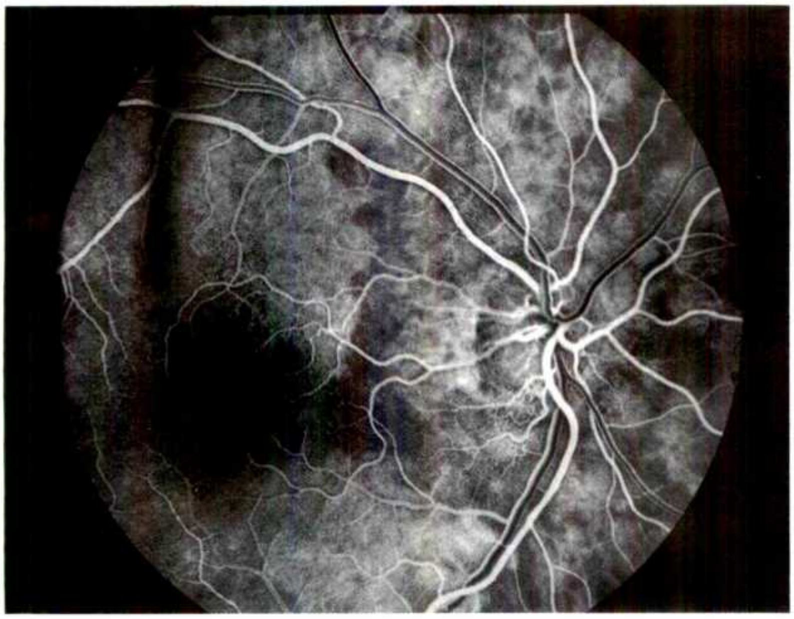

Figure 2 Fluorescein angiography of the right eye, early arteriovenous phase. Inferotemporal capillaries are visible.

right optic nerve. Repeated laboratory investigations were again normal. An electrocardiogram was also normal.

Thirteen days later vision deteriorated further on the right to 6/12, N14, and 2/17 Ishihara plates. Fundus appearances were unchanged but Goldmann perimetry showed a right central scotoma. In view of this, and the suggestion of abnormal vessels on the right disc mitochondrial DNA (mtDNA) studies were undertaken to investigate the possibility of LON as described by Holt *et al.*[1]

Despite treatment with acetazolamide 2×250 mg/day, followed by prednisolone 80 mg/day and intravenous dextran, visual loss continued in a stepwise manner over 1 month. On 15 May 1991 visual acuity of the right eye was 3/60, N36, with absent, red-green colour vision. The right disc swelling had subsided and Goldmann perimetry showed enlargement of the central scotoma. Full-field pattern-reversal VEPs were undetectable in both eyes.

During this period of deteriorating vision in the better eye, optic nerve sheath decompression was considered in view of encouraging reports in progressive ischaemic optic neuropathy,[2] but genetic analysis revealed the point mutation at position 11778,[3] which was present in more than 95% of blood, mtDNA confirming the diagnosis of LON for which surgery provides no benefit.

Discussion

Leber's optic neuropathy (LON) often exhibits maternal inheritance and a possible underlying mtDNA mutation was suggested.[4] Three mutations found exclusively in LON families have been reported to date.[1 3 5 6] Typically LON affects mostly adolescent or young adult males and is rare over the age of 50. It is characterised by painless bilateral loss of central vision, the interval between involvement of the two eyes being rarely more than a few months. In the acute stage the involved optic disc may show hyperaemia with peripapillary microangiopathy but without fluorescein leakage, and progresson to optic atrophy is usual.[7-10]

Worldwide, more than 50% of LON pedigrees show a point mutation of mtDNA at position 11778. This results in substitution of histidine for arginine in subunit 4 of complex 1 of the mitochondrial respiratory chain.[2 3 11] Our patient presented at 63 years of age with bilateral optic neuropathy with involvement of the second eye almost 1 year after the first. The age of onset with inferior field loss to a red target, and the absence of a relevant family history were in favour of an ION.[12 13] The time course suggested a progressive form of this condition. However the central scotoma and slightly swollen optic disc with a suggestion of peripapillary telangiectasia without fluorescein leakage were sufficiently suggestive of LON for the genetic analysis to be performed despite the unlikely clinical setting.

The inferior peripapillary telangiectasia may have been within normal limits but, in association with the visual loss, was attributed to the capillary dilatation seen with resolving disc oedema in ION. This raises, therefore, the importance of considering LON in any patient with telangiectasia and central visual loss.

Recently the clinical characteristics of 72 patients with LON and the 11778 mutation were reported.[11] Though not differing from the classic description of LON[8 9] with regard to age at onset, visual acuity, and male predominance typical fundus findings were detected in only 30/52 patients (58%), no patients demonstrated ECG abnormalities and, most importantly, 28 of 49 pedigrees consisted of isolated cases. Such findings contrast markedly with previous reports.[14] Indeed delayed onset LON has been reported in patients up to 70 years of age[15] but this has almost always occurred in the context of a family history of blindness or within families with typical cases of LON or with asymptomatic carriers.[14 15]

MRI of the optic nerve using a STIR sequence revealed a lesion in the left but not in the right optic nerve. The lesion in the left optic nerve extended over 10 mm and was mostly posterior within the orbit. Such a length and location are more frequently seen in LON than in demyelinating optic neuritis,[16] and have never been reported to occur in association with ION. Despite a clinically bilateral optic neuropathy only the left optic nerve showed a lesion on MRI. Such a discrepancy has been reported by Kermode *et al* who found that 3/7 patients with bilateral LON had a unilateral optic nerve lesion on MRI.[16]

Genetic studies showed the 11778 base pair mutation in more than 95% of mtDNA. This mutation has not been detected in more than 300 controls.[11] Therefore despite the lack of family history and the delayed onset of visual loss we believe our patient presented with late onset isolated LON. Without genetic testing and MRI scanning the aetiology would probably have been attributed to ION which is the most frequent cause of optic neuropathy after the age of 60 years and bilateral involvement varies from 35% to 50%.[13]

Diagnostic confusion may occur with chronic atypical optic neuropathy and this paper emphasises the value of modern genetic and radiological investigations.

We thank Mr R Dewhirst for photographic help. The genetic studies were performed in Professor A E Harding's laboratory.

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British Journal of Ophthalmology 1992; **76**: 574–576

Concurrent uveoretinitis and pineocytoma in a child suggests a causal relationship

Niels Illum, Horst W Korf, Kim Julian, Tommy Rasmussen, Margrethe Herning, Søren Krabbe

Department of Pediatrics, Hvidovre Hospital, Copenhagen, Denmark
N Illum
S Krabbe

Center of Morphology, Johann-Wolfgang-Goethe University, Frankfurt, Germany
H W Korf

Department of Ophthalmology, Hvidovre Hospital, Copenhagen, Denmark
K Julian
T Rasmussen

Danish Research Center of Magnetic Resonance, Hvidovre Hospital, Copenhagen, Denmark
M Herning

Correspondence to:
Dr N Illum, Department of Pediatrics, Hvidovre Hospital, Kettegaard Allé 30, DK-2650 Hvidovre, Denmark.

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Abstract

Uveoretinitis was observed in a 9-year-old girl 6 months prior to the clinical appearance of a pineal tumour. Surgical removal was not successful but biopsy revealed a parenchymal neoplasm with differentiated pinealocytes and absent mitotic activity. Some of the tumour cells contained S-antigen, rhodopsin, and serotonin. Systemic glucocorticoid therapy followed by radiation therapy caused considerable reduction in size of the tumour and a complete normalisation of all eye symptoms. This report demonstrates for the first time that a pineocytoma can occur together with uveoretinitis in humans. The latter resembles the experimentally induced autoimmune uveoretinitis described in animals. It is speculated that the retinitis might reflect an autoimmune response to S-antigen present in some tumour cells of the pineocytoma.

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The retina and the pineal organ of all vertebrates, including humans, share several molecules and proteins.[1,2] One of these is the highly antigenic S-antigen located in the retinal photoreceptors and pinealocytes.[1,2] When administered in small amounts to susceptible animals the S-antigen induces an inflammatory response in the uveal tract, retina, and pineal gland.[2] This is considered as a paradigm to clarify the nature and mechanism of inflammatory eye disease in humans.[3] However, it has not yet been established whether immunological eye diseases in humans coincide with pathological changes in the pineal organ or vice versa. We report a patient suffering from a pineocytoma associated with uveoretinitis and suggest a causal relationship between both symptoms.

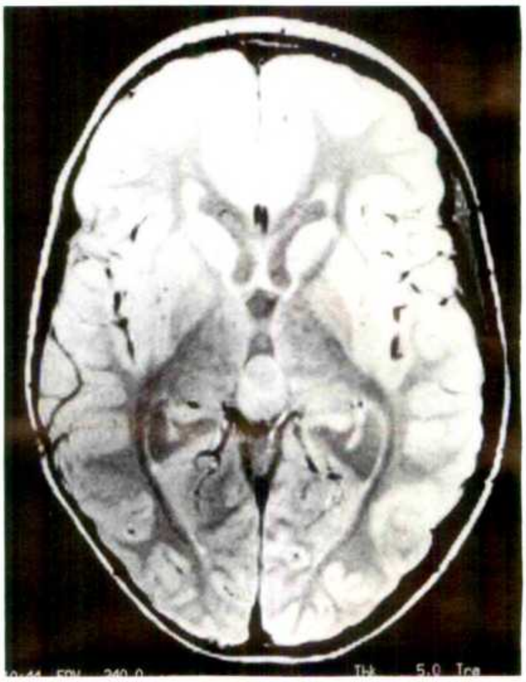

Figure 2 Magnetic resonance imaging studies showing the pineocytoma before radiation therapy.

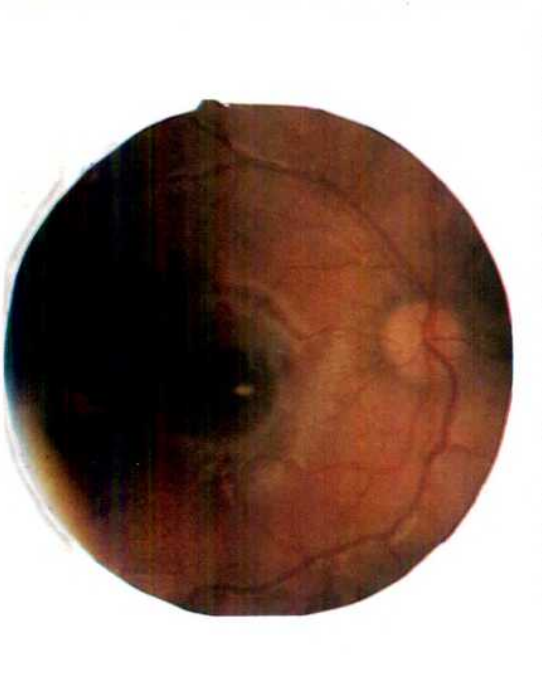

Figure 1 Funduscopic examination of the right eye. Central oedema is present.

Materials and methods

CASE REPORT

A 9-year-old otherwise healthy Caucasian girl presented with bilateral iritis followed 3 months later by a retinal oedema in the central fovea of the right eye. Cycloplegics and topical corticosteroids cleared the inflammation which, however recurred upon subsequent attempts to reduce the corticosteroid doses. Eight months after referral a sudden reduction of vision was experienced. The optic nerve was pale, swollen, and protruded 2 dioptres. Iritis reappeared together with retinitis, presenting as intraretinal macular oedema (Fig 1) and intraretinal oedema in the periphery of the retina of both eyes. Retinal vasculitis or retinal pigment epithelial detachment was not evident by repeated ophthalmic examinations in this young girl. Campimetric examination disclosed bitemporal visual field defects. Magnetic resonance imaging showed hydrocephalus and a pineal tumour obstructing the aqueduct (Fig 2). Systemic glucocorticoid therapy and a shunt operation completely reversed the symptoms. Radiation therapy was administered and 1·5 years later the gland had returned to a normal size. Ophthalmic examinations remained normal even after the glucocorticoid therapy had been stopped during radiation. Tissue typing showed that the patient carried antigens HLA-A28 DR4.

HISTOLOGY

The pineocytoma consisted of small, dark, and round nuclei arranged in groups and with occasional rosette formations. Mitoses could not be detected (Fig 3).

IMMUNOCYTOCHEMISTRY

Paraffin sections were used for the demonstration of S-antigen, rhodopsin, and serotonin. The S-antigen antibody (raised in rabbits) was a generous gift from Dr I Gery, National Eye Institute, Bethesda, MD, USA. The rhodopsin antibody (raised in rabbits) was kindly provided by Dr W J de Grip, Department of Biochemistry, University of Nijmegen, Nijmegen, The Netherlands. Both antibodies have been well characterised.[4-8] The serotonin antibody was purchased from IBL, Hamburg, Germany. All antibodies were diluted with phosphate buffered saline (PBS) containing 1% bovine serum albumin and 0·3% Triton X-100 (Sigma, St Louis, MO, USA) to a final concentration of 1:1000. Binding was visualised by the peroxidase-antiperoxidase method.[4,9]

IMMUNOLOGICAL ANALYSIS OF CSF SAMPLES

CSF samples were obtained 19 days after the shunt operation and processed for SDS-polyacrylamide gel electrophoresis.[10] The proteins were then electroblotted onto nitrocellulose, incubated with the S-antigen antibody (diluted 1:4000) and binding was visualised by the peroxidase-antiperoxidase method.

Results

HISTOLOGY

Immunohistological examination for the presence of T cell markers LCA, 4KB5, L-26, CD3 and UCHL-1 was negative.

IMMUNOHISTOCHEMISTRY

S-antigen, rhodopsin, and serotonin immunoreactions were observed in certain tumour cells scattered among immunonegative elements which formed the major portion of the tumour. S-antigen appeared evenly distributed in the cytoplasm and was absent from the cell nuclei (Fig 4a). Rhodopsin immunoreaction was mainly demonstrated in the periphery of the cells (Fig 4b). The rhodopsin immunoreactive cells were less frequent than those containing immunoreactive S-antigen. The serotonin immunoreaction was evenly distributed in the cytoplasm or concentrated in the periphery of certain cells (Fig 4c) whose number and distribution closely resembled those of the S-antigen immunoreactive cells.

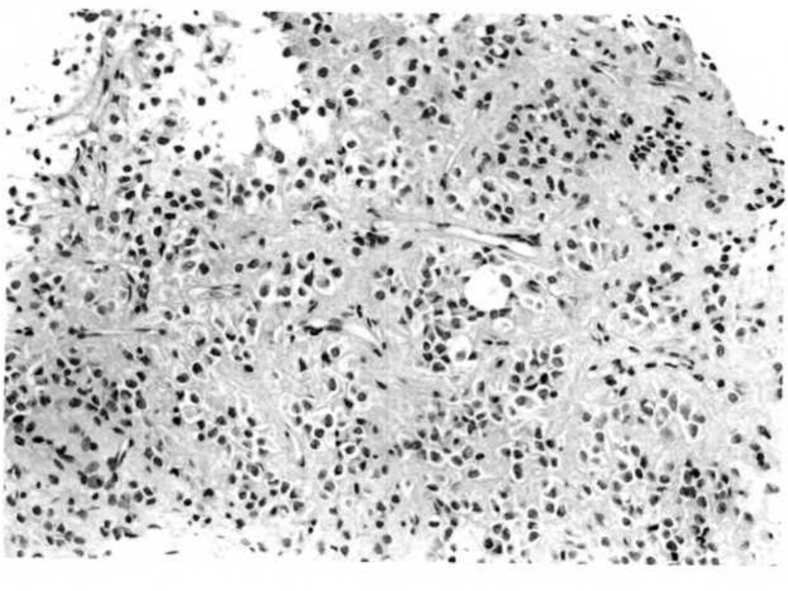

Figure 3 Pineocytoma composed of cells with small, dark and round nuclei. Occasional rosette formation is noted. Mitoses could not be detected. ×85.

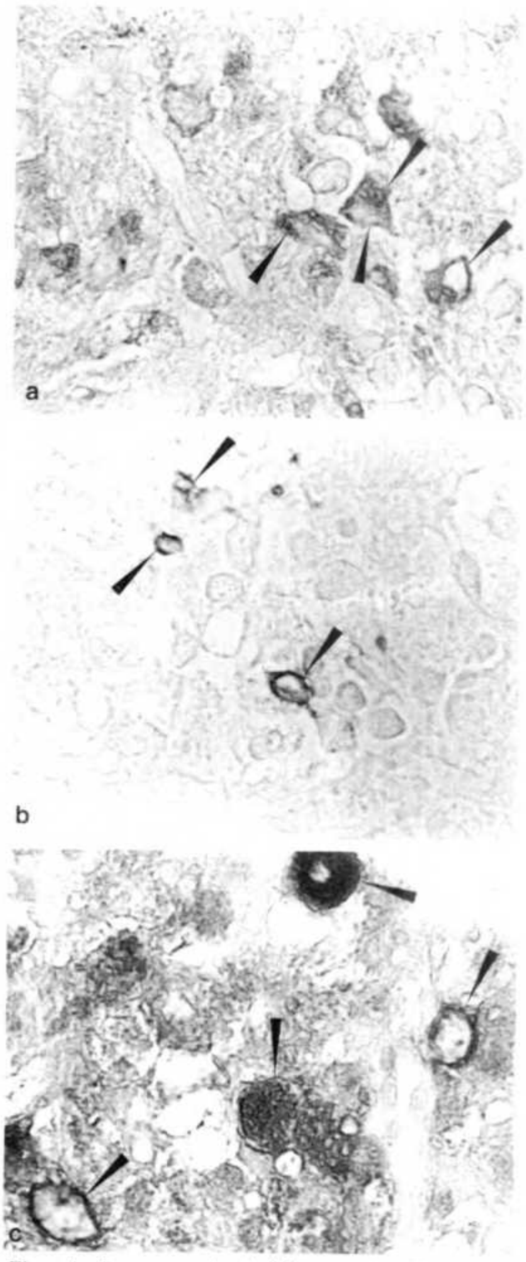

Figure 4 Immunocytochemical demonstration of S-antigen (a), rhodopsin (b), and serotonin (c) in paraffin sections of the tumour biopsy. Arrowheads indicate immunoreactive cells, a, b ×350, c ×480.

IMMUNOCHEMICAL ANALYSES OF CSF SAMPLES

Immunochemical (western blot) analysis failed to demonstrate a protein band reacting with S-antigen antibody.

Discussion

We report a 9-year-old girl suffering from uveoretinitis and a tumour of the pineal region classified as a pineocytoma by histological features and demonstration of S-antigen, rhodopsin, and serotonin immunoreactions in some tumour cells.[5 10 11] The simultaneous occurrence of uveoretinitis and a pineal tumour, and the disappearance of all ophthalmic manifestations upon tumour therapy suggest that both symptoms may be related and resemble those found in experimental autoimmune uveoretinitis with associated pinealitis. The autoimmune reaction, which has an anterior as well as a posterior component, is shortlived and disappears when the provoking factor is eliminated.[2]

Though we cannot explain exact methods of immune stimulation – the apparent absence of T cells from the tumour and S-antigen from the CSF[10] – the presence of S-antigen in tumour cells might still be an important pathogenetic factor. Moreover considering the presence of S-antigen in certain cerebellar medulloblastomas,[7] it is surprising that S-antigen containing tumours have not presented with uveitis in the past. An association with certain antigens determined by the major histocompatibility complex could play a role. The Vogt-Koyanagi-Harada syndrome is associated with HLA-DR53 in Japanese but with HLA-D4 in American patients.[12] Our patient had DR4. Other immune mechanisms involved could be the way uveitogenic epitopes of the S-antigen are presented to the immune system,[2] selective involvement of pathogenic T cells with a restricted T cell receptor V gene usage,[2] or an inappropriate suppression of an autoimmune response elicited.[13]

Irrespective of these unresolved problems, we conclude on the basis of the clinical experience obtained from the care of this child that a tumour of the pineal gland should be included in the differential diagnosis of uveitis in childhood. These tumours often have a more malignant course in children than in adults,[14] and symptoms in the eye could precede the classic symptoms of an expanding and obstructing pineal process by several months.

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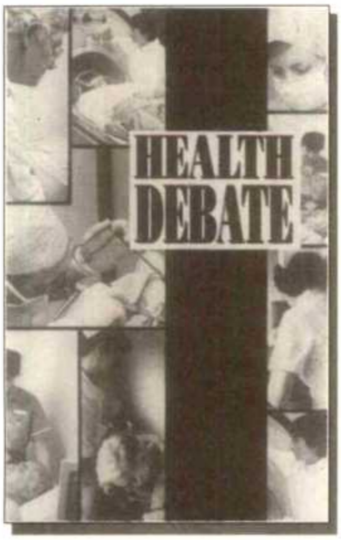

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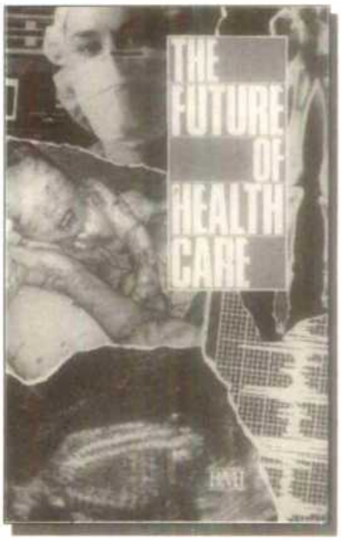

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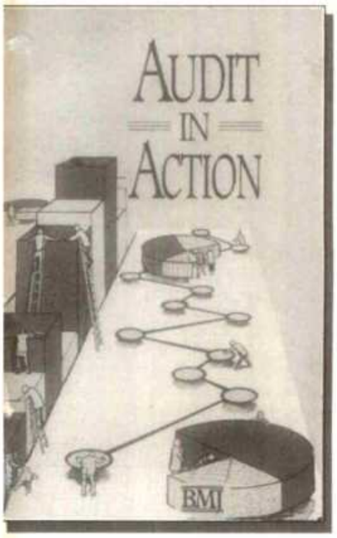

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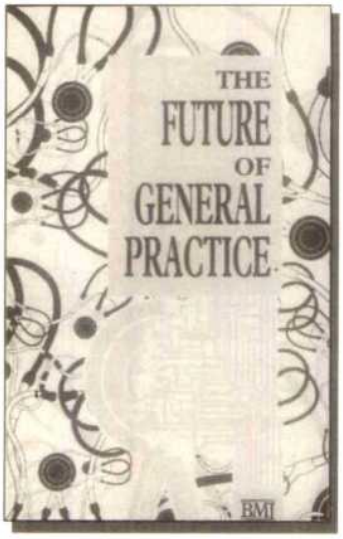

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